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STUDIES ON THE SEASONAL PREVALENCE AND DISPERSAL OF THE EGYPTIAN HOUSEFLY[1] [2]

Part II. The Larvae and Their Breeding Areas

R. T. HOLWAY, LCDR MSC USN, W. A. MITCHELL, HMC USN, AND ABDEL AZIZ SALAH

United States Naval Medical Research Unit No. 3, Cairo, Egypt

INTRODUCTION

Part I of this report was concerned with the seasonal abundance and dispersal of the adult flies. Part II takes up the problem of fly breeding areas, their location and seasonal changes in degree of activity.

Elimination of the breeding foci has long been recognized as the most satisfactory method of fly control. When this is not possible or practical the usual procedure is to attempt control by mechanical, biothermic or chemical means. In recent years great emphasis has been placed on the search for newer and more effective methods and insecticides. However the discovery of new techniques which greatly increase the effectiveness and efficiency of chemical applications against adults is still unlikely to mean that the problem of fly breeding can be neglected.

The typical Egyptian peasant (fellahin) village provides great amounts of favorable fly media. Living standards and habits and agricultural practices are such that effective control by conventional sanitary methods alone would involve basic and far-reaching changes in the lives and work of the people, changes which are considered unlikely in the foreseeable future. It may be for this reason that too little attention has been given to an analysis of the types of places and materials which produce flies in Egyptian villages beyond general references to manure heaps, latrines and stables.

A purpose of this study was to determine in more detail some of the actual areas and foci where fly breeding occurs most frequently

[1] The opinions expressed in this article are those of the authors and are not to be construed as reflecting the views of the Navy Department.

[2] The authors wish to express their gratitude for the assistance received during the course of this work from the staff of the U. S. Naval Medical Research Unit No. 3.

and to classify in so far as possible the important types of areas in order that planning and discussion of possible coordination of sanitary and chemical control methods might be facilitated.

A thorough understanding of the fly producing foci and the relationship between the presence of these foci and the daily routines of the villagers might give rise to new ideas on ways in which slight changes in living habits and agricultural practices would eliminate much of the breeding without the requirement of revolutionary and impossible changes.

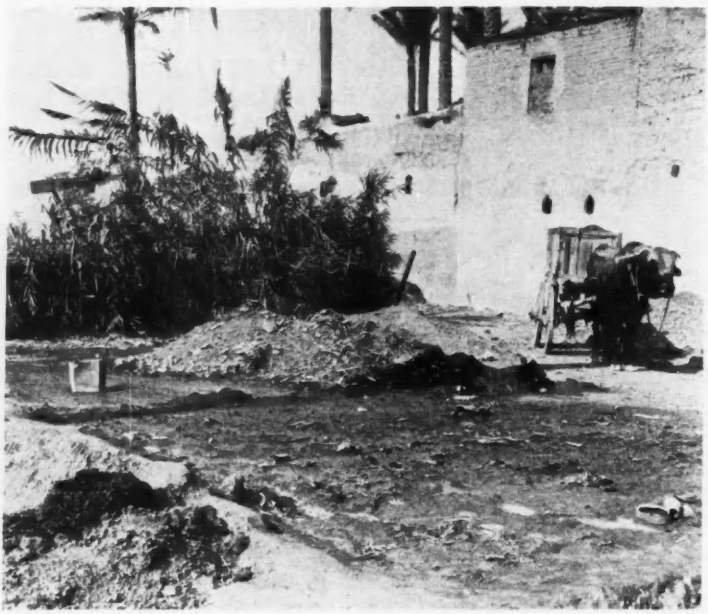

FIG. 1. A typical latrine-dump consisting of waste organic materials, human excreta and waste water.

It is believed that a survey such as this will be useful as a background for future detailed studies which will be necessary and which may well cover many years before the fly problem in Egypt is solved.

TYPES AND DISTRIBUTION OF BREEDING AREAS

Observations made in four typical villages (Ashtar, Moneeb, Wali Bey and Harania) of Giza Province during the latter part of 1948 indicated that four main types of fly breeding areas are readily distinguished. These were named latrine-dumps, compost or manure piles, animal rooms and fuel cakes. A miscellaneous class was also

designated to include the many smaller fly producing foci which are less clearly defined.

Latrine-Dump

The latrine-dump is an area where human feces and other organic wastes are habitually deposited. Figures 1–4 illustrate typical examples. The material shown in fig. 1 is accumulated over a period of time by continual dumping of waste organic matter, feces and water. A large proportion of the mixture consists of human dung removed from cesspits in the houses.

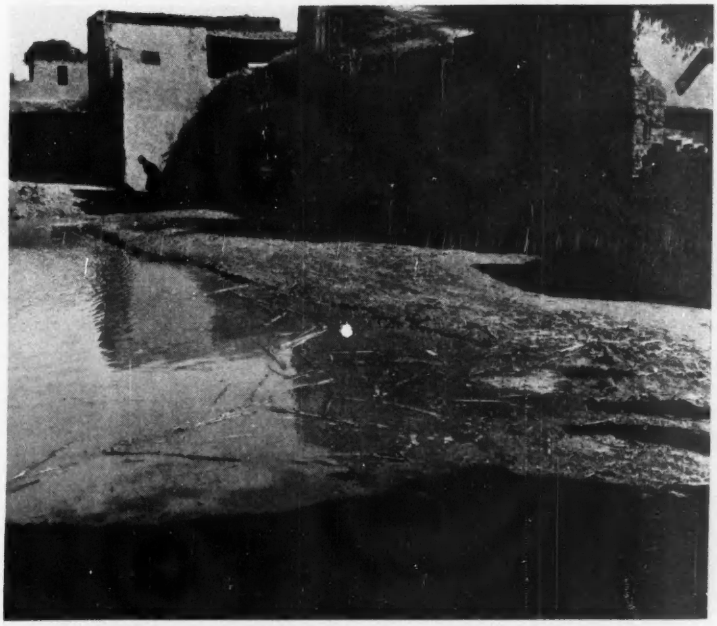

FIG. 2. Latrine-dump area at the margin of a pond.

Areas such as this must exist because there are no sewage systems to carry off the wastes and in any case the material is too valuable to the farmer as a fertilizer to be discarded. When the mixture of cesspit contents, organic refuse and soil reaches a depth of several inches or is required as fertilizer the material is dug out and carried to the fields and a new accumulation begins.

Figure 2 represents a common variation of the latrine-dump area. At one or several points along the margins of a pond or canal organic refuse and cesspit contents may be dumped and the blended mixture of soil, garbage, human feces and cornstalks is usually found to be teeming with fly larvae.

Figure 3 illustrates the use of the sides of an old drainage ditch for a dump area. The area in the foreground is composed mainly of garbage, human feces and organic refuse. Waste water from several families is continuely thrown out on these areas during the day so that the moisture content of the mixture is maintained during the driest seasons.

Another type of latrine-dump, fig. 4, is provided with required moisture by the overflow from the water pump. Garbage and human dung are deposited in the low area below. Quantities of wastes from a milk and cheese shop are discarded at the right and provide an attractive and prolific breeding area.

FIG. 3. Latrine dump formed by discard of garbage, human feces and waste water along an old drainage ditch.

Some of the latrine-dumps are clearly defined, individually owned and purposely developed as a source of fertilizer. Others are merely waste disposal areas which originate by chance and are not owned or utilized as planned sources of manure. All of these various types of latrine dumps are found within the village and although there is a tendency for many to be located near the outer limits and along pond and canal margins they also occur in the open squares and along the village streets adjacent to the houses.

Compost Pile

The second main type of fly breeding area is the compost pile, fig. 5, so characteristic of the village economy. Nearly every family with animals owns one or more of these piles, which grow by daily additions of dung from the animal room. Typically the process is as follows: a pile of dry soil from the fields is maintained near the house and each day a few basketsfull of the unmanured soil are spread around the animal stand within the house. Within a day or two the accumulated dung, usually gamoose or donkey, with occasionally human, mixed with the soil, is collected in baskets and is dumped on the compost pile. This continues over a period of weeks or months until

FIG. 4. Overflow from water pumps often provides moisture required to make fly production centers of dump areas.

the enriched soil is returned to the fields, usually at a time when a certain crop is to be planted. If the pile of fertilizer is not used by the owner it is sold and in either case is an important part of the peasant's assets. The compost piles increase in size and number throughout the autumn, winter and spring until April when a high percentage is used during corn planting.

Note in figure 5 the mounds deposited on the main compost pile. The new material maintains the shape of the basket in which it was

carried and normally fly breeding is found only in these collections of fairly recently deposited material. The main body of the compost pile is generally too old and dry to support fly larvae.

Breeding material added to the compost pile is not always in the form of the dense well defined mounds described above. Sometimes a loose mixture of straw and donkey dung is spread over the heap in a thin layer and may be heavily infested while the main body of the pile beneath this layer remains negative. Human dung, garbage and organic wastes may also be deposited on the pile in small amounts although in most cases this type of material is thrown on the latrine-dump.

FIG. 5. Compost pile. Fly breeding occurs most often in the small, recently deposited mounds on top of the main pile.

Animal Room

The animal stands or rooms, Figure 6, constitute a third category of fly breeding area. The majority of families in the villages surveyed maintain one or more animals which are stalled in one room of the house.

The types of dung which make the animal room a breeding area are the same as those found on the compost heaps. As described above, the bulk of the animal droppings are removed daily but a certain amount always remains and becomes worked into the soil and litter. The combination of dung, soil, refuse and urine provides a suitable

breeding medium at one or several foci in the animal room. The accumulated material which forms the floor of the animal room is removed after a period of several months and is utilized as fertilizer. Figure 6 (foreground) illustrates how one half has been removed to a depth of several inches while in the background a new accumulation is developing.

The entire area of the animal room does not breed flies uniformly but, as in the case of the compost piles, only at certain points where dung or other material of a suitable type, age, consistency and moisture content is present.

FIG. 6. Animal room. Fly breeding occurs only at scattered points where suitable materials are present.

Fuel Cakes

Fuel cakes comprise a fourth major fly breeding category. The high cost of ordinary fuels in Egypt has forced the villager to utilize even his animal dung for cooking and heating. The dung of the buffalo is mixed with straw and formed into flat discs about one inch thick, which are dried in the sun (figs. 7, 8). Another type (fig. 8) of fuel cake is made in the form of a narrow bar, hollowed on one side to facilitate stacking by nesting one on another. These are stored, usually on the roofs, as shown in figures 2 and 4.

As a rule only one to three day old buffalo dung is used although small amounts of other types of dung may occasionally be included. The women and older children collect the dung, mix it with chopped straw and form the typical discs or bars. Note the hand prints in figure 7. According to Hafez (1941) these discs dry completely in two or three days in the summer and in five or six days in winter, and he points out that length of time before the dung is made up into fuel cakes is an important factor in the importance of these as a fly breeding source. If oviposition occurs after the cakes are formed the

FIG. 7. Fuel cakes made from buffalo dung and straw.

larvae do not have sufficient time to develop and pupate before the disc is completely dried out. On the other hand if the larvae are several days old when the cakes are formed they may complete development, migrate into the soil and pupate below the disc. The bar-type cakes are less susceptible to desiccation as they are somewhat thicker and when stacked they retain sufficient moisture for seeds to sprout.

Miscellaneous

Numerous other fly breeding foci occur which have not been classified separately because of infrequent occurrence, lack of consistent and uniform distribution or rare presence of breeding. The total contribution of these foci to the fly population however may be very great.

Individual deposits of human feces occur throughout the village; usually these are found at common defecation areas along a wall or around a latrine-dump area. Fly larvae are very often found in these deposits and during periods of high humidity development is completed. However fly emergence from these foci during the hot, dry seasons is probably not common.

Cesspits or bore-hole latrines are found in from five to ten percent of the houses. In most cases the contents are in a liquid condition because of a high water table and their contribution to the fly population

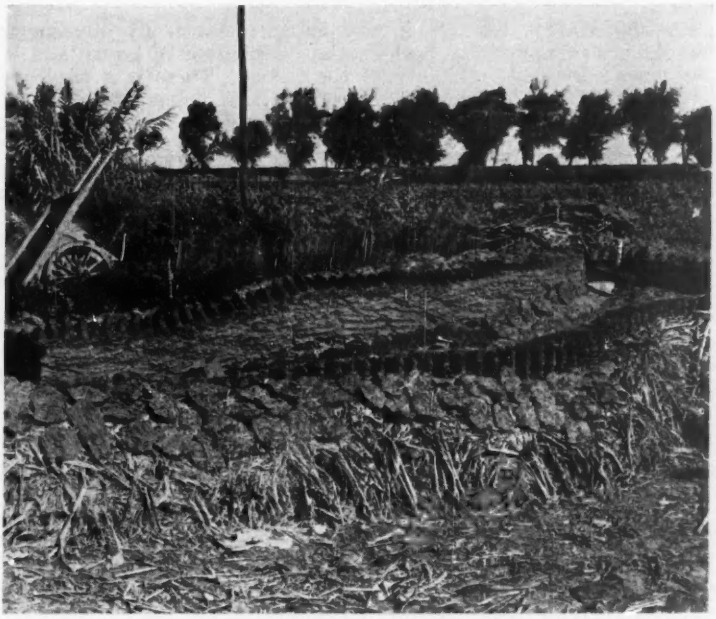

FIG. 8. Fuel cakes, disc and bar types.

probably varies with local conditions. Fowl pens where droppings are allowed to accumulate in a large mass are potential fly producers but usually fowl dung is too well scattered to be an important factor. Fly larvae are occasionally found in mud, dung and straw mixture which are used for the production of building bricks. Small collections of garbage, vegetable wastes, blood soaked soil and stomach contents at slaughtering sites often become breeding foci but as a rule material of this type is located on a typical latrine-dump.

The four main areas which comprise this working classification are defined more from a functional standpoint than from a consideration of the types of dung present. That is, the relationship between the

presence of certain definite fly breeding foci and the daily routines and practices of the people is considered, on the basis of this survey, to be the most important factor in any program to reduce the fly breeding potential by cultural or sanitary methods.

BREEDING FREQUENCY

The above classification was compiled from data collected during the latter part of 1948. A survey procedure was then developed and standardized to determine what seasonal variations occurred, if any, in the relative breeding activity of the different types of areas throughout 1949.

A fundamental difficulty in any measurement of fly breeding is the absence of uniformity both in the distribution of larvae and in the consistency and suitability of the media. There is a tendency for the adult flies to oviposit in groups so that many larvae will be found at one point while a few feet away in what is apparently the same media none are found. Estimation of the cubic footage or square footage of breeding material is another problem. For example the amount of potential fly media in a large compost pile may vary from 0% to 100% of the total cubic contents of the pile. The procedure followed here does not pretend to measure actual amounts of fly breeding in the different type areas. The numbers of sites within which evidence of fly breeding is found is the unit of measurement from which an index is derived.

Check stations were designated at scattered locations throughout four villages. These stations were selected as far as possible so that typical examples of latrine-lumps, compost piles, animal rooms and fuel cakes would be represented. Fifteen stations were chosen at Ashtar, eleven at Moneeb, eleven at Wali Bey and ten at Harania. At each station, each of the four types of breeding areas, if present, was checked once every week for the presence of fly larvae or pupae. The types of dung or organic material present were listed in order of the most common. The same foci were checked each week so that seasonal rather than locality differences would be reflected in the totals. Approximately the same amount of time was allowed for searching each latrine-dump and animal room station before it was listed as negative for breeding. Four compost piles and ten fuel cakes were checked at each station before it was reported negative.

The number of units checked is an arbitrary choice because there is no basis for estimating equivalent breeding potential, that is, there is no basis for considering that one compost pile is equivalent to one fuel cake or any other fixed number. However if equal numbers of units are checked in successive weeks any real increase or decrease of total larvae present will increase or decrease the likelihood that evidence of breeding will be found and hence that a positive station will be recorded. Four compost piles and ten fuel cakes were selected as the standard for each station merely because it seemed that these numbers would be sufficient to assure enough positive findings each week for a working percentage. Checkers were trained to search only material where fly breeding could reasonably be expected to occur. In other words material that was obviously too dry or too deficient in organic material was not included.

The weekly or monthly breeding index for each type area derived from these observations is merely the percent positive of the total number of stations examined. Variation in the relative number of active breeding sites from season to season is revealed by this survey. Also any insecticide treatment which is effective enough to eliminate all detectable breeding in a significant number of the check stations should be reflected in a lower breeding index.

Before attempting to analyze the significance of the seasonal changes in the breeding index it is necessary to review some of the important factors which affect fly breeding.

ENVIRONMENTAL FACTORS WHICH AFFECT FLY BREEDING

Temperature

There is a great deal of information on the effect of temperature on muscoid fly metabolism but for present purposes it is only necessary to emphasize that high as well as low temperatures have a retarding effect on fly activity and development. Melvin (1934) found that the incubation period of the egg of *M. domestica* was prolonged by high temperatures. Deonier (1942), on the basis of field studies and mean temperatures, reported that *Lucilia sericata* Meig. and *Phormia regina* Meig. were rapidly reduced in numbers by monthly mean temperatures above 21° C, whereas the optimum range for *Cochliomyia macellaria* (F.) was from 21° C to 26.8° C.

From a study of the results of Lorincz and Makara (1936) in Hungary and adult fly abundance in Egypt (*cf.* Part I) it appears that the most favorable mean monthly temperatures for houseflies are approximately between 20° C and 25° C. As monthly mean temperatures rise much above 25° C there is a retarding effect while in the lower ranges moderate activity continues down to 15° C with a more rapid drop in abundance apparent as temperatures fall from 15° C to 10° C. Since these are mean temperatures they are of course lower than the actual daytime temperatures which influence fly dispersal.

The effect of temperatures on fly dispersal may also be a factor in the activity of certain types of breeding areas. It has been found in Egypt that flies move from the outside to the inside of village houses as temperatures rise from 25° C to 30° C (*cf.* Part I). During June, July and August the fly population becomes more concentrated inside the houses and the population density outside is very low (fig. 11).

Moisture Conditions

Dakshinamurty (1948) found that maximum housefly activity occurs with high temperatures of low humidity and minimum activity with high temperatures and high humidity. At low temperatures with either high or low humidity activity is intermediate in degree. He concludes that this principle may explain in general the greater fly nuisance in hot dry climates as compared with those of moderately high temperatures and high humidity.

On the other hand lower humidities would be expected to bring about some reduction in total amount of suitable breeding media by a higher evaporation rate.

Rainfall and ground water are important where they provide moisture not otherwise available to breeding materials. Most of the latrine-dump areas receive sufficient water from daily household waste, pump drains and pond or canal margins. Winter and early spring rains may contribute to the moisture supply of compost piles and fuel cakes.

The percentage of stations checked which were reported as moist or slightly moist for three types of breeding areas during 1949 are shown in figure 13. The animal rooms have the lowest percentage of "moist" stations during April, May and June when relative humidity is also at its lowest points for the year. Similarly the animal rooms show the highest percentage of "moist" stations during October, November and December when relative humidity is also highest. Latrine dumps and compost piles are most often dry in June, July and August when rainfall is practically zero (fig. 12) and temperatures are highest (fig. 10).

Attractivity of Media

A great many investigators of housefly breeding have commented on the types of media in which flies oviposit. ***Musca domestica*** and ***Musca domestica vicina*** have been reported consistently to prefer horse, pig, and human dung to cow or buffalo. Recent evidence indicates that human feces is even more attractive to ***vicina*** than has been realized generally. A breakdown of the compost pile breeding index may be significant in this respect. Table I shows how the presence of human excrement results in a breeding index more than double that found when only animal dung and garbage are present.

Quarterman *et al.* (1949) reports on the percent of premises positive for fly breeding in Savannah, Georgia. Examinations during the active season revealed that 90% of privies, 60% of garbage containers and from 7% to 41% (depending on locality) of dog feces were producing flies. Although the conditions are not comparable it is of interest to note that the high frequency for human excrement is consistent with the data obtained in Egyptian villages. The 60% figure for garbage is substantially above the percentages found in Egyptian villages where garbage appeared to be no more attractive than animal dung alone. Discarded food wastes are relatively uncommon in the villages since practically all nutrient materials, especially those containing protein, are utilized either by humans or animals and this may account for the low attractivity of such garbage as may be present. The only possible conclusion from these various observations is that human excrement is extremely attractive to the common Egyptian housefly and particularly so to the ovipositing female.

The work of Hafez (1941) indicates that certain species or subspecies prefer cow and buffalo dung. This raises an interesting problem in the field of morphological versus biological variants, but from the medical standpoint species which comprise only a small percentage of the total fly population and are not attracted to human feces are not of major importance.

An important consideration from the control standpoint, however, is the extent to which the common feces-visiting species, ***M. domestica vicina*** breeds in buffalo dung. According to Hafez ***M. vicina*** rarely

or never breeds in cow or buffalo dung unless it is mixed with other types. The breeding index for fuel cakes (Table II), made to a great extent from buffalo dung, is very high in all four villages during the summer and fall. Thus it appears that at least a portion of the village fly population originates from fuel cakes during part of the year. The fact that 97.5% of 1,222 flies of the genus *Musca* collected by the authors in June, July and August, 1948 were *M. domestica vicina* and only 2.5% were *M. sorbens*, with none of the other *Musca* represented (Sabrosky, *in litt.*) indicates that flies other than *vicina* were most uncommon during the summer in these villages. From the control standpoint it is important that further work be undertaken to determine conclusively whether *M. domestica vicina* breeds to any extent in fuel cakes and if not what percent of the total fly population is made up of the other species which do, and to what extent they visit human excrement.

TABLE I

BREEDING INDEX FOR 1949 OF COMPOST PILES AT FOUR EGYPTIAN VILLAGES

TYPE OF BREEDING MATERIALS PRESENT	PERCENT OF STATIONS POSITIVE FOR BREEDING			
	VILLAGE			
	Ashtar	Moneeb	Wali Bey	Harania
Animal dung only	37	32	26	28
Animal dung and garbage	33	32	16	22
Animal dung and human	73	65	66	52
Total stations checked	695	462	529	516

Population Densities

The magnitude of the adult fly population will obviously have a profound effect on the extent of fly breeding and on the types of material which are infested. Only the most favorable sites are likely to be utilized when adult fly populations are below the capacity of the breeding media available whereas when populations are in excess of the capacity of breeding media oviposition may occur in all possible sites and even where development cannot be completed (Scudder, 1949).

The amount of potential breeding media present in Egyptian villages is so great that in most cases it is likely that factors other than population pressure are more important in determing where fly breeding occurs. An exception probably occurs when the great majority of flies are concentrated inside of houses.

ANALYSIS OF SEASONAL FLUCTUATIONS

Latrine Dumps

The breeding index of the latrine-dumps (fig. 9) is at its lowest point of the year in February. The winter low of both the adult density index (fig. 11) and the breeding index coincide with the low mean

monthly temperatures of 11.1° C and 11.3° C for January and February (fig. 10). Moisture is plentiful during this season and it is quite obvious that temperature here is the governing factor.

During March the mean temperature rises above 15.0° C and breeding frequency starts to increase and climbs rapidly to a high level in April and May. The adult index also rises sharply but lags somewhat behind the breeding index. The highest levels of the year are reached in the season when all factors are at the optimum. Mean monthly temperatures are between 16.0° C and 25.0° C and rainfall, although light, does occur through April. Relative humidity is low and favorable to maximum activity at the higher temperature in May yet probably not so low as to contribute to serious dessication. It is not surprising to find the annual peak in fly abundance during this season when all factors are at an optimum. From May to August the latrine-dump index falls consistently. Examination of the meteorological data (figs. 10, 12, 14) reveals several unfavorable trends. No measurable rainfall occurred after April and mean temperatures were above 25.0° C during June, July and August. Outside fly populations dropped sharply during late May and June and became extremely low in July and August. The number of "moist" latrine-dump areas is lower during June, July and August (fig. 13) but the amount of suitable breeding material of this type available is certainly far greater than can be fully utilized by the very low outside adult population.

The question arises whether the lower breeding frequency index is due primarily to the effects of the unfavorable temperatures or to moisture conditions. An attempt has been made to eliminate the moisture factor by omitting the totally dry locations in calculating the percent positive. No actual measurements of moisture content were made so that a few of the areas included in the total may not have been suitable for fly breeding. However, the number of such cases could not be sufficient to alter greatly the frequency index as the latrine-dump areas by definition have an obvious source of moisture as well as organic material and excreta. The dry areas were eliminated because they no longer gave evidence of any moisture supply and therefore were not counted as true latrine-dumps.

In any case the fact that the number of suitable, well watered latrine-dumps which were positive dropped to less than 50.0% during the summer indicates that some factor other than moisture is responsible for the lowered fly population. If moisture were the governing factor it would be expected that as the total area of favorable breeding sites contracted due to lack of ground moisture a higher percentage of the remaining favorable sites would become infested by the relatively greater population pressure and consequent utilization of all favorable media. Eventually, when the capacity of breeding sites to produce flies was reached, a leveling off of the adult population would be expected. Actually the adult population does not level off but drops very rapidly (fig. 11), coincident with the rise of monthly mean temperatures above 25.0° C. This occurs in June in the case of outside population while the inside population continues to increase for another month, due to the movement of flies from outside to inside of the houses. The outside fly density has dropped to nearly zero and the inside density to 60.0%

of the June level by July. The fall in the breeding frequency index is what would be expected from a greatly reduced adult population and an abundance of suitable breeding material.

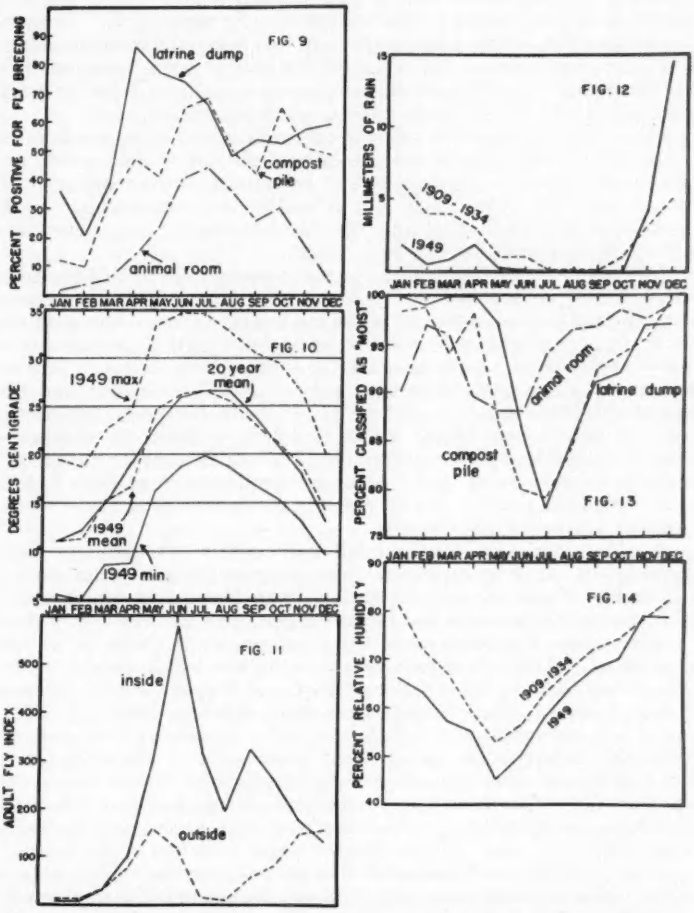

FIG. 9. Breeding index for latrine dumps, compost piles and animal rooms at Ashtar. FIG. 10. Temperatures at Giza Province. FIG. 11. Adult fly index at Ashtar. FIG. 12. Rainfall at Giza Province. FIG. 13. Percent of breeding areas classified as moist. FIG. 14. Percent relative humidity at Giza Province.

All factors considered it appears that the one most important is the high temperatures acting directly by decreasing the activity of the adults and probably by increasing larval and adult mortality and

indirectly by driving adult flies indoors for the greater part of the day so that they have less opportunity for oviposition in latrine-dumps. There is no question but that the total number of latrine-dumps suitable for breeding is decreased during the hot, dry summer, and this may contribute to some extent to the smaller total fly population. However the fact that the breeding frequency index of even *suitable* latrine-dumps is so much lower shows that all available media is not being utilized. For this reason the moisture factor appears to be less important than temperature and the dispersion of the adult population.

The latrine-dump index begins a secondary and more gradual rise in September which continues throughout the fall. Both inside and outside adult counts also rise again in September as the mean monthly temperature falls below 25.0° C. A satisfactory explanation for the much lower level of fly breeding frequency during the fall as compared with the spring peak has not been found.

Temperatures are favorable and great quantities of suitable breeding material are present making it even more difficult than in summer to show that lack of moisture is the governing factor. The relatively low adult populations of March and early April with favorable temperatures were able to nearly saturate the latrine-dump breeding areas as evidenced by an April frequency index of 86.0% with resultant very high adult densities in May and June. If the total amount of potential breeding material in latrine-dumps had been reduced by absence of rainfall to a low level in September and October, then the limited amount of media should be even closer to saturation inasmuch as adult flies are more numerous than in March and temperatures are in the optimum range.

Another indication that rainfall may not be the primary factor affecting fly breeding in latrine dumps is the absence of an increase in breeding frequency and adult density in November commensurate with the November rains and mean temperatures above those of April.

Here again, the *dispersal* of the adult population may be of some importance. Although figure 11 shows outside adult counts in September and October below those of April and May the actual difference is even greater. These counts were made between 1100 and 1300, a period of maximum activity during late September, October and November, whereas the spring counts were made at the same time of day and do not show the much higher levels which occur before 1000 (*cf.* Part I). The maximum temperature curve in figure 10 reveals how lower temperatures comparable with spring temperatures which favor outside fly concentration do not occur until late in the fall.

This distribution of the adult flies may not be the full explanation of the lower breeding frequency at latrine-dumps and the smaller adult populations during the fall months. The possibility that some other factor is involved which has not been studied can not be ignored. For example the accelerating effect of low winter temperatures on the rate of development of grasshopper eggs (Parker, 1930) is suggestive. The monthly mean minimum temperatures for March and April (25 year average) at Giza are 7.9° C. and 10.4° C. and for September, October and November they are 17.6° C. 15.3° C. and 12.2° C. The lower minimum temperatures during the spring indicate that a study of the

possible accelerating effect of temperatures on housefly development may be of interest. Another possibility may be more widespread occurrence of fly diseases during the fall.

Compost Piles

The compost pile breeding index (fig. 9) increases from its low winter level less rapidly than the latrine-dump index and instead of reaching a peak in April or May, continues to rise until July. In view of the fact that the material of the compost pile in which fly breeding is actually found is, in most cases, that which has recently been removed from the animal rooms, it is probable that much of the oviposition occurs within the animal rooms before the dung is removed to the compost pile, as well as outside after it is deposited on the piles.

During the spring when outside fly populations are high the compost piles must compete with the much more attractive latrine-dumps for the oviposition of the outside adult population while inside adult populations are relatively low. However, from May on, as flies tend to concentrate more and more inside, the situation is reversed and increased oviposition, even though in the less attractive animal dung, would be expected due to the greater population pressure inside the houses. In the relatively small number of cases in which human dung is found mixed with the compost pile material the percent positive is much higher as would be expected from the greater attractiveness (Table I). By August the adverse effects of high temperatures have operated for a sufficient time to bring down even the inside fly population with a resultant decline in the breeding index.

Moisture as ground water seems to be even less a factor in the case of the compost piles than that of latrine-dumps. The necessary moisture is obtained in most cases from that contained in the dung originally. It would be expected that evaporation rate and original moisture content would determine whether or not the material remained sufficiently moist long enough for the larvae to develop to pupation.

The breeding rate is actually highest when temperatures are highest and relative humidity is at its annual low points (May, June and July), which is also the period when the percentage of compost piles classified as "moist" is the lowest. This is most probably explained by the inside concentration of adult flies since the breeding index of compost piles follows the decrease of the inside adult population and rises again soon after inside fly concentrations begin to increase. The situation during the fall however is complicated by the occurrence of rains, higher humidities and a trend to higher outside fly densities so that outside oviposition may be increased also. The absence of any marked drop in the compost pile breeding index until January could be the net result of these interacting factors.

Animal Rooms

The animal room breeding index (fig. 9) follows a course similar to that of the compost piles but with a slower increase in the spring and a more rapid decline in the fall. This would be expected since the animal rooms are entirely dependent on the inside adult population for oviposition while compost piles may benefit from both inside and outside

adult populations. In this connection it may be significant also that the animal room index during the fall is generally somewhat higher than the spring level. This is consistent with the higher inside adult puoplations found in the fall as compared with the spring inside densities.

Breeding surveys were also carried on at the three other villages and similar seasonal trends were found. Notable variations from the Ashtar seasonal curve occurred only after insecticide treatments at these villages which were applied in connection with another project.

Summarizing the relationship between inside and outside fly breeding and inside and outside adult populations we find the following general picture:

1. March-April—As temperatures progress into favorable and optimum ranges breeding in the outside, very numerous, highly attractive areas increases rapidly and produces a tremendous fly population. Low inside adult populations oviposit to a limited extent in the less attractive materials available inside so that breeding indices rise more slowly.

2. May-June—A tendency for flies to concentrate more and more inside is associated with the rising temperatures. The greatly increased and concentrated adult fly population would be expected to produce more oviposition inside with a consequent rise of compost pile and animal room breeding indices while latrine dump indices fall as outside populations become low. The higher temperatures also must be effecting a progressive decline in total numbers of flies present by decreasing adult activity and oviposition and increasing larval and adult mortality or by forcing oviposition in less favorable inside materials or by a combination of both effects.

3. August—The persistent high temperatures have brought about by August a marked decrease in adult populations both inside and outside with a resultant drop in breeding frequency in all areas. During the last two weeks of August temperatures drop into the optimum range which should initiate increased activity and lower mortality thus foreshadowing increased adult counts in September.

4. September-October-November—Favorable temperatures and increased adult populations produce only moderate or erratic increase of the breeding frequency index throughout the fall in the case of latrine-dumps and compost piles but a falling index for the animal rooms. A staisfactory explanation for the absence of fly densities comparable with those of the spring is not evident from the available data. It is not believed that rainfall is a primary factor. An unbalanced distribution of adult flies to the inside due to the effect of temperature in such a way that latrine-dumps do not receive enough oviposition to fully utilize their capacity for producing enormous fly populations until late November or December is a suggested cause. The accelerating effect of low winter temperatures or lower night temperatures in March and April is another possibility.

5. December-January-February—There is a general drop in all fly activity as monthly mean temperatures fall into unfavorable ranges.

Fuel Cakes

The part which fuel cakes play in fly production is not clear. The breeding index shows considerable variation but there is a definite

tendency for higher percentages to occur during September, October and November (Table II). Apart from the unfavorable low winter temperatures relative humidity appears to be the most important factor which affects breeding frequency in fuel cakes. The much higher relative humidities of the fall and lower evaporation rates would permit more time for development. This would be expected as the fuel cakes are particularly susceptible to rapid drying.

The villagers say that, "worms" are found in fuel cakes only when the buffalo are fed on "dry feed." During December, January and February the buffalo are fed on green clover, in March, April and May on both green clover and dry feeds of various kinds, and during the remainder of the year on dry feeds only. Larvae have been found in fuel cakes at all seasons but it may be that when the buffalo are not fed on green clover the fuel cake mixtures are more attractive or favorable for fly breeding. This requires further investigation.

TABLE II

SEASONAL VARIATION IN BREEDING INDEX OF FUEL CAKES
AVERAGE OF FOUR VILLAGES

Month	Temperature Conditions	Mean Relative Humidity	Type Feed	Breeding Index
January	Cold	66	Green	2
February	Cold	63	Green	0
March	Medium	57	Both	51
April	Optimum	55	Both	41
May	Optimum	45	Both	42
June	Hot	50	Dry	72
July	Hot	58	Dry	64
August	Hot	64	Dry	73
September	Optimum	68	Dry	84
October	Optimum	70	Dry	81
November	Optimum	79	Dry	88
December	Medium	79	Green	51

Table II lists the average breeding index of four villages along with temperature conditions, relative humidity and types of buffalo feed. The most that can be concluded is that when the index is highest (September, October and November) the relative humidity is at its seasonal peak, temperatures are in the optimum range and the buffalo are on "dry feed."

More complete taxonomic studies are needed to determine whether the flies produced by this material, or a significant portion of them, are the same as those produced by other types of breeding areas.

Miscellaneous Breeding Foci

No surveys were made of breeding frequency in the miscellaneous types of fly producing media as most of these are not numerous enough to contribute an important part of the total fly population. The total contribution of all these foci however may be considerable.

Separate, individual human stools may produce a very high percentage of the fly population when evaporation rates are not high enough to thoroughly dry out this material before pupation is accomplished.

APPLICATIONS

The most significant condition disclosed by this survey is the high frequency with which materials containing human excreta are found to be producing flies in Egyptian villages. A second important feature is the part which moisture plays in maintaining suitable breeding areas. This factor does not appear to operate uniformly for all types of breeding areas since latrine dumps are affected to a less extent by low rainfall and humidity and are highly dependent on "man-made" moisture sources. On the other hand breeding in individual human stools, compost piles and fuel cakes appears to be affected to a considerable extent by relative humidity, rainfall and evaporation rates.

A point of attack in the case of many of the latrine dump type areas may be the moisture supply. Separation of waste water and waste organic materials would eliminate much of the breeding potential at least during the rainless months. Simple but tight drains to carry household waste water and pump overflows to ponds or irrigation system drains would prevent waste water from contributing to the breeding potential. A more difficult problem would be to prevent dumping along the margins of ponds and canals. Concentration of dumping in well defined, recognized areas instead of in widespread, unorganized and changing areas as at present would facilitate sanitary control and where necessary the application of chemicals. Organized dumps should be sufficiently numerous and convenient to keep resistance to their use at a minimum. At the same time the number of such areas should not be greater than can be properly supervised and treated when necessary by a village sanitarian.

The possibility and advisability of reducing the necessity for chemical treatments by "composting" the night soil accumulations according to the Indore or Calcutta methods (Roy, 1946) should be considered. However the careful supervision and attention required and the problem of individual ownership of small accumulations may be factors against the use of these methods.

The compost piles are dependent more on moisture contained in the original materials and evaporation rates, than on waste or ground water. The compost pile is such an integral part of the village economy that its elimination is probably impossible and even concentration of the material would be impractical due to the ownership problem.

It has been suggested that oviposition could be prevented by covering the material with a layer of dry earth. This might be a useful practice if the fellahin could be prevailed upon to carry it out conscientiously. A more serious objection however is the probability that a significant amount of oviposition occurs before the material is removed from the animal rooms.

If the newly deposited material could be broken up and spread out over the surface of the compost piles daily much of the breeding might be eliminated by desiccation during a large part of the year.

There would probably be many instances and periods when chemical applications would be required but such treatments should be far more effective when applied to a thin raked-over layer than to the usual dense, mound-like deposits. Responsibility should be fixed with a village sanitarian rather than left to the individual owners of the piles.

Minor and immediately practical changes in customs and habits or sanitary procedures which might reduce fly production in human stools, other than collection and concentration, are not readily apparent. Reduction of fly breeding in fuel cakes may be accomplished if the dung is formed into cakes before oviposition takes place according to Hafez (1941). Changes in shape and thickness of the fuel cakes and periodic turning to increase evaporation should also be investigated as possible measures to reduce breeding.

There have been numerous reports in recent years on the relationship between sanitation and the effectiveness of chemical treatments. An insecticide application which is highly effective at one level of abundance for fly breeding may be overwhelmed by tremendous fly populations when there are great quantities of suitable fly media. Thus any major decrease in fly breeding should contribute to the usefulness of chemicals. Large scale applications of some of the new insecticides to breeding areas has given excellent fly control in Egyptian villages for limited periods under experimental conditions. The efficiency of such methods could be greatly increased by a consideration of which areas are the most important fly production centers. Elimination of breeding foci wherever possible and concentration of breeding materials in organized dumps to facilitate insecticide applications should contribute to reduced expense.

Continued and more detailed study of the fly breeding areas in conjunction with social, economic and sanitary advances would certainly contribute towards the development of more effective methods for the reduction of breeding potential in all types of areas.

Summary

The common types of fly breeding areas found in Egyptian villages are described in detail and classified according to their occurrence in terms of the habits and daily routines of the people. Four distinct areas are distinguished: latrine-dumps, compost piles, animal rooms and fuel cakes. A fifth miscellaneous category includes the more scattered individual human stools or infrequent foci such as fowl pens and building-mud mixtures.

Relative seasonal changes in the breeding rates of the four main areas were determined by weekly surveys. Latrine-dumps were found to be producing flies most often in April and May, compost piles and animal rooms in June and July and fuel cakes during the fall months. All classes except fuel cakes show a decline in August. During the fall latrine-dumps and compost piles show a moderate rise while animal rooms decrease steadily. All areas decline in December and reach an annual low point in February.

The seasonal variations in breeding frequency are interpreted in terms of environmental factors. The interaction of temperature,

adult population density and dispersion, and the high attractiveness of human feces, was found to be most important in determining the seasonal activity of latrine-dumps, compost piles and animal rooms. Moisture, as rainfall and relative humidity, appears to be least important in the case of latrine-dumps and animal rooms but favorable to increased breeding frequency in compost piles and particularly in fuel cakes and human stools.

Moisture, as household waste water, pump drainage, and pond and canal margins, is a highly important factor in maintaining the breeding potential of latrine-dump areas. Separation of this type of moisture supply from accumulations of organic materials is suggested as a possible approach to reduction of breeding potential at latrine dumps. Daily raking over of the top layers of compost piles to increase evaporation may be a worthwhile procedure. Coordination of sanitary methods with chemical methods would produce increased effectiveness and economy in village control programs and continued study of fly breeding areas in relation to local customs and habits is essential.

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NORDENS EUPITHECIER, by KNUD JUUL. 147 pages, 13 plates (6 in color), 48 maps, 10 text figures. Gravers Andersens Forlag, Aarhus, Denmark. 1948. Price, $4.75.

This very attractive little book describes, illustrates, and maps the distribution of 56 species of adults, larvae and pupae of *Eupithecia*, *Gymnoscelis*, and *Chloroclystis* (Lepidoptera, Geometridae) that occur in Denmark. The text is both Danish and English. The geographical distribution of the species into Norway, Sweden, and Finland is also tabulated. Adults, larvae and sometimes pupae are shown in color, and the genitalia are illustrated in line drawings. The book is attractively though simply bound, the paper of high quality, the arrangement excellent, and the illustrations well executed and attractively reproduced. The book is a work of art.—M. T. J.

MINERAL NUTRITION OF THE TWO-SPOTTED SPIDER MITE, TETRANYCHUS BIMACULATUS HARVEY

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Relatively little is known of insect nutrition and even less has been reported on the nutrition of the closely related mites. Since minerals are among the basic nutritional factors and cannot be synthesized by an organism as can some nutrients, any mineral essential to the organism must be obtained from exogenous sources. The availability of the minerals therefore influences the metabolism, and in turn, the resulting population.

McGarr (1942) found that nitrogenous fertilizer caused a definite increase in the number of cotton aphids. Evans (1938) found a positive correlation between the young produced by *Brevicoryne brassicae* L. and the amount of nitrogen in the plant. Haseman (1946) reported that while the grain aphid bred more uniformly on full nutrients, deficiencies in the supply of phosphorous, sulfur, potassium and magnesium had no apparent ill effect on reproduction; however, a nitrogen deficiency effected poor reproduction and survival.

Dahms and Fenton (1940) concluded that the resistance of two varieties of milo sorghums to chinch bug attack was consistently decreased by sodium nitrate and increased by superphosphate added as fertilizers. Dahms (1947) found that chinch bugs laid more eggs on sorghum plants growing in solutions high in nitrogen or low in phosphorus than on the same varieties growing in solutions low in nitrogen or high in phosphorus. Haseman (1946) reported, however, that the chinch bug lived longer and produced more offspring on corn plants when nitrogen was withheld from the nutrient solution. Wittwer and Haseman (1945) found that thrips did not injure spinach grown in high levels of nitrogen (on clay-sand cultures) but badly injured the spinach grown at the lower nitrogen levels. When the calcium supply was increased, the attacks on the low nitrogen groups were less serious.

Loeb (1915) demonstrated that *Drosophila* require phosphorus for larval development. Nelson and Palmer (1935) reared *Tribolium* on a synthetic ration containing five levels of phosphorus and found that on higher phosphorus levels a shorter time was required for pupation. Hobson (1935) found that blowfly larvae did not attain optimum growth on a diet of blood and serum unless phosphorus and potassium was added.

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[2]These studies, which formed part of the author's doctorate thesis, were conducted in the Department of Entomology of the Ohio Agricultural Experiment Station under the direction of Dr. R. B. Neiswander, Associate Entomologist, and Dr. D. M. DeLong, Professor of Entomology at the Ohio State University. The thesis, now on file at The Ohio State University, contains detailed data involving methods and analyses not included here. Grateful acknowledgment is made of the assistance of Dr. J. D. Sayre, Physiologist at the Ohio station.

In investigations conducted in Ohio, it was found that a decrease in soil soluble salts over a three-year period was accompanied by a decrease in populations of the two-spotted spider mite, *Tetranychus bimaculatus* Harvey, infesting tomatoes in commercial greenhouses (Rodriguez and Neiswander, 1949). A further survey disclosed that the commercial fertilizer applications in these houses had been reduced by approximately 36 percent over a five-year period (Rodriguez, 1949–50). Lending support to these findings was the work of Garman and Kennedy (1949) who found that a heavy fertilizer program on beans and peaches increased two-spotted spider mite populations. In one series, nearly three times as many mites developed on the fertilized plants as on the unfertilized.

In the present study, it was desired to ascertain the influence of a single mineral, or combination of minerals, on the development of this mite. Tomato plants were grown in nutrient culture in a series of mineral levels, and mites were introduced onto the host plants and allowed to develop on isolated foliage. The criterion of measurement was the response of these mites to their diet, as determined by the resulting number of progeny developing over a given period of time.

Since exposing plants to a series of mineral levels does not imply that mineral uptake is proportional to mineral supply, it was deemed desirable to analyze the foliage for minerals in order to consider the inorganic nutrition of the plant as one of the variables. In this paper, a report is given of the influence of both supply and absorption of nitrogen, phosphorus, sulfur, magnesium, potassium, calcium and various levels of a standard nutrient solution on the mite population.

Although it was recognized that the organic phase of plant nutrition is undoubtedly an important factor in the nutrition of insects or mites that suck plant juices, this phase was not investigated in these experiments. It was also recognized, however, that the organic phase of plant nutrition is basically dependent on the inorganic phase.

EXPERIMENTAL

Nutrient Culture Studies

Three-gallon glazed crocks were used to grow tomato plants, with about 40 pounds of quartz gravel in each crock to support the plant. Each crock was connected to a 13-liter reservoir of nutrient solution, which was maintained at that level with soft (rain) water. Irrigation was automatic.

A complete solution which was considered optimum for growing tomatoes was used as a standard solution or treated control in each test. This solution was composed of the following chemicals at the indicated molar concentrations: KH_2PO_4 at .000645 M; KNO_3 at .005 M; $Ca(NO_3)_2$ at .005 M; and $MgSO_4$ at .0021 M. Variations stemmed from this standard. To test a specific mineral, that particular ion was the only one varied over a series of concentrations. Iron, furnished as finely-ground magnetite mixed in the gravel medium, and a complete minor element solution suggested by Sayre *et al.* (1940) were also common to all the experiments. Solutions were replaced every two weeks.

Globe (Strain A) tomato plants were used throughout the experiments. When the plants were setting fruit at the third cluster, leaflets adjacent to this cluster were prepared for introduction and isolation of the mites. Leaflets were selected uniformly according to age, size, position relative to direction, location within a leaf, and distance from a fruit cluster. Each stem was wrapped with a short strip of waxed paper and cellulose tape, over which a tanglefoot barrier was applied with a hypodermic syringe. Thus the leaflet was effectively isolated and prepared for the introduction of mites. Twelve or 14 leaflets from each plant were isolated and infested during the course of the test but three or four were usually infested at a given time on each plant.

The mites used throughout this work came from a culture that has been maintained in the entomology greenhouse for a period of years. Five young adult female mites were selected and transferred on bean leaf sections to each isolated leaflet, where they developed; the resulting progeny were counted at the end of approximately thirty days. Observations made frequently on the hatchability of the eggs and the sex ratio indicated that the eggs were virtually all viable. (Unfertilized females produce only male progeny.)

The major elements could not all be tested concurrently; the studies required three experiments conducted over a period of approximately eighteen months. In the first experiment, four concentrations of nitrogen (100, 200, 400 and 800 ppm.) and three of phosphorus (5, 20 and 40 ppm.) were tested. Four concentrations of sulfur (6, 60, 150 and 300 ppm.) and three of magnesium (5, 50 and 125 ppm.) were tested together in the second experiment. In the last experiment, four concentrations of potassium (20, 200, 500 and 1000 ppm.), three of calcium (20, 200 and 500 ppm.), and four levels of the standard solution, varied in its entire concentration (0.2, 1.0, 2.0 and 4.0 times) were tested. (The concentrations in parts per million are expressed here in round figures for the sake of convenience; some ion levels deviated slightly from these numbers.) One of the concentrations of each mineral was supplied by the standard solution which was included in all the experiments. Each mineral level was replicated four times.

Foliage Analyses

A sample of 100 leaflets was taken from each plant. The samples were dried, ground, mixed and stored in tightly capped sample bottles until analyzed.

The Kjeldahl method was used to determine nitrogen (Anon., 1940). Sulfur determinations were made on the samples from the sulfur study, using the method of Evans and St. John (1944).

Phosphorus, potassium, calcium, magnesium, sodium, iron, boron, manganese, silicon, aluminum and copper were measured spectrographically. The general methods applicable to the quartz spectrograph outlined by Brode (1943), Harrison *et al.*, (1948), and Sayre (1944) were followed.

A medium quartz Bausch and Lomb spectrograph was employed; excitation was accomplished by using a 2200 volt AC arc at 2.2 amperes. Purified graphite rod electrodes were used.

The percentage ash was determined for each sample; the amount

of ground tissue which would produce 10 mgs. ash was calculated and added to 90 mgs. of lithium fluoride buffer. The mixture was muffled at 800° F. for two hours, ground, and arced in duplicate arcings. The spectral lines were photographed on "Eastman 33" plates and read in a non-recording densitometer.

A synthetic tomato ash, based on chemical analyses of tomato foliage samples (Howlett, 1949) was prepared and analyzed in a series of concentrations with the buffer. From the readings of these concentrations, the working curve for each mineral was plotted.

TABLE I

MEAN MITE POPULATIONS THAT DEVELOPED ON TOMATO PLANTS GROWING IN FOUR LEVELS OF NITROGEN SUPPLY, AND MINERAL ANALYSES OF TOMATO FOLIAGE AS CORRELATED WITH POPULATIONS

POPULATION AND MINERAL ELEMENT	PERCENT MINERAL ABSORBED (DRY WT.)[1] ON LEVELS OF NITROGEN (PPM.)				CORRELATION	
	100	200[2]	400	800	*r* value	Level of significance
Nitrogen	3.27	3.61	4.43	6.11	—0.657	.01
Phosphorus	.204	.144	.376	1.388	—0.632	.01
Potassium	3.39	3.72	4.30	2.82		
Calcium	9.40	6.68	3.76	1.75	+0.565	.05
Magnesium	1.06	.86	.61	.33	+0.572	.05
Sodium	.189	.132	.165	.344		
Iron	.081	.017	.066	.033		
Boron	.028	.024	.042	.160	—0.683	.01
Manganese	.0041	.0019	.0019	.0019		
Silicon	.355	.221	.324	.396		
Aluminum	.278	.133	.155	.191		
Copper	.026	.019	.049	.047		
Total Ash	26.4	23.3	20.4	15.7	+0.690	.01
Mean Mite Population[3]	62.4	53.9	53.0	19.2		

[1]Mean of four replicates.
[2]Standard solution level.
[3]Mean progeny of 12 series of 5 female mites each; minimum significant difference between means: 17.6, 1 percent level; 13.4, 5 percent level.

The densitometer reading of one of the lithium lines was used as a standard throughout this work. The densitometer readings of the lines were calculated to absolute values in percent of tomato leaf tissue, dry weight basis.

Variations in mite population which might be ascribable to mineral level (treatment), influence of plant age (series), and the interaction of these two main sources were tested in an analysis of variance, multiple classification. Differences in population were found on replications of the same mineral level; similarly, differences in mineral absorption occurred in replications. To determine whether variations in population were related to variations in mineral absorption, tests for correlation coefficient *r* were made.

RESULTS

Mineral Supply and Mite Population

There were great differences in the size of the populations which developed on the various treatments of the nitrogen and phosphorus experiment. There was also a small variation due to series. However, analysis of variance showed that treatment was responsible for the major portion of variation in populations. The upper level of nitrogen (800 ppm.) produced a much smaller population than the other three

TABLE II

MEAN MITE POPULATIONS THAT DEVELOPED ON TOMATO PLANTS GROWING IN THREE LEVELS OF PHOSPHORUS SUPPLY, AND MINERAL ANALYSES OF TOMATO FOLIAGE AS CORRELATED WITH POPULATIONS

POPULATION AND MINERAL ELEMENT	PERCENT MINERAL ABSORBED (DRY WT.)[1] ON LEVELS OF PHOSPHORUS (PPM.)			CORRELATION	
	5	20[2]	40	*r* value	Level of significance
Nitrogen	3.07	3.61	3.01		
Phosphorus	Trace	1.44	Trace	+0.692	.05
Potassium	4.67	3.72	4.49	—0.691	.05
Calcium	6.67	6.68	6.75		
Magnesium	.96	.86	.92		
Sodium	.223	.132	.206	—0.615	.05
Iron	.018	.017	.023		
Boron	.028	.024	.030		
Manganese	.0037	.0019	.0037	—0.775	.01
Silicon	.122	.221	.163		
Aluminum	.186	.133	.223		
Copper	.027	.019	.033		
Total Ash	24.3	23.3	24.4		
Mean Mite Population[3]	16.8	53.9	10.7		

[1]Mean of four replicates.
[2]Standard solution level.
[3]Mean progeny of 12 series of 5 female mites each; minimum significant difference between means: 17.6, 1 per cent level; 13.4, 5 per cent level.

levels (Table I). In the three phosphorous levels, the standard solution level was responsible for a greater population than either the low or high level (Table II).

Variations attributable to mineral level and plant age were noticeable in the mite populations which developed on the sulfur and magnesium experiment. Neither factor, however, showed significance in the analysis of variance tests. Considering the minimum significant differences between treatment means, it will be seen that the lowest level of sulfur (6 ppm.) produced the highest population and that this population was highly significant from that produced on the standard

solution level (60 ppm.) only (Table III). The highest level of magnesium (125 ppm.) produced a population significantly higher than that produced by the standard solution (50 ppm.) (Table IV). The low level was responsible for a population which was not significantly different from either of the others. In this experiment the error remained relatively high. A number of female mites introduced did not produce progeny; this study was conducted over the winter months, and it is possible that the culture may have undergone unfavorable conditions even though the greenhouse temperature was controlled.

TABLE III

MEAN MITE POPULATIONS THAT DEVELOPED ON TOMATO PLANTS GROWING IN FOUR LEVELS OF SULFUR SUPPLY, AND MINERAL ANALYSES OF TOMATO FOLIAGE AS CORRELATED WITH POPULATIONS

POPULATION AND MINERAL ELEMENT	PERCENT MINERAL ABSORBED (DRY WT)[1] ON LEVELS OF SULFUR (PPM.)				CORRELATION	
	6	60[2]	150	300	*r* value	Level of significance
Nitrogen	3.98	3.70	3.31	3.48		
Phosphorus	.128	.071	.213	.095		
Sulfur	.64	1.80	2.51	2.37		
Potassium	4.78	4.29	4.44	4.59	+0.669	.01
Calcium	6.21	6.86	9.05	8.14		
Magnesium	1.83	1.11	1.51	1.22	+0.698	.01
Sodium	.207	.171	.205	.181	+0.604	.05
Iron	.027	.023	.034	.021		
Boron	.040	.024	.052	.025		
Manganese	.0006	.0007	.0005	.0004		
Silicon	.290	.153	.265	.185	+0.819	.01
Aluminum	.221	.141	.196	.166	+0.839	.01
Copper	.015	.014	.011	.011		
Total Ash	21.3	24.0	26.9	26.1		
Mean Mite Population[3]	31.0	13.0	22.0	19.6		

[1]Mean of four replicates.
[2]Standard solution level.
[3]Mean progeny of 14 series of 5 female mites each; minimum significant difference between means: 16.3, 1 percent level; 12.4, 5 percent level.

The potassium, calcium and standard solution experiment had a relatively small error. Treatment was responsible for the major portion of the variations in population. The general influence of potassium supply on population was that of a positive correlation; *i.e.*, the mite population increased as the potassium concentration was increased (Table V). The 1000 ppm. level produced a population which was highly significant from those which developed on the 20 and 200 ppm. levels.

Calcium supply appeared to have no significant effect on mite development even though the concentration ranged from 20 to 500 ppm. (Table VI).

The relationship of various levels of the standard solution to mite population was an interesting one (Table VII). There was no marked change in population when the concentration of the salts was increased from 0.20 to 1.0 times the optimum level. When this concentration was doubled (2.0), the population was also doubled. This increase in population was significantly different at the one percent level from any population produced on the other levels of the standard solution.

TABLE IV

MEAN MITE POPULATIONS THAT DEVELOPED ON TOMATO PLANTS GROWING IN THREE LEVELS OF MAGNESIUM SUPPLY, AND MINERAL ANALYSES OF TOMATO FOLIAGE; (POPULATIONS NOT SIGNIFICANTLY CORRELATED WITH ANALYSES)

POPULATION AND MINERAL ELEMENT	PERCENT MINERAL ABSORBED (DRY WT)[1] ON LEVELS OF MAGNESIUM (PPM.)		
	5	50[2]	125
Nitrogen	3.65	3.70	3.56
Phosphorus	.049	.071	.177
Potassium	4.87	4.29	4.17
Calcium	8.18	6.86	6.57
Magnesium	.63	1.11	2.19
Sodium	.164	.171	.183
Iron	.030	.023	.012
Boron	.042	.024	.054
Manganese	.0011	.0007	.0003
Silicon	.229	.153	.257
Aluminum	.188	.141	.144
Copper	.010	.014	.013
Total Ash	24.8	24.0	23.3
Mean Mite Population[3]	20.9	13.0	26.8

[1]Mean of four replicates.
[2]Standard solution level.
[3]Mean progeny of 14 series of 5 female mites each; minimum significant difference between means: 16.3, 1 percent level; 12.4, 5 percent level.

Mineral Absorption and Mite Population

Nitrogen

The influence of variations of total nitrogen on the mineral composition (both major and minor elements) of the tomato foliage was profound (Table III). Nitrogen showed a steady increase; quite in contrast was the effect on calcium, magnesium and ash. Potassium and sodium varied only slightly. Phosphorus decreased slightly when nitrogen was increased from 100 to 200 ppm., but rose sharply when the nitrogen levels were increased further.

The potassium content of foliage was decreased on the upper level of nitrogen. The 100 and 200 ppm. levels were furnished as nitrates while ammonia nitrogen was added to the 400 and 800 ppm. levels. It seems likely that there was interionic competition between the ammonium and potassium ions, causing the decrease in potassium absorption. This agrees with the findings of Dikussar (1934). Phosphorus showed a tremendous increase with the higher increments of ammonium. This also agrees with Dikussar's report. Calcium was depressed; Jacobson and Swanback (1933) demonstrated the effect of ammonium in depressing the absorption of calcium in tobacco.

TABLE V

MEAN MITE POPULATIONS THAT DEVELOPED ON TOMATO PLANTS GROWING IN FOUR LEVELS OF POTASSIUM SUPPLY, AND MINERAL ANALYSES OF TOMATO FOLIAGE; (POPULATIONS NOT SIGNIFICANTLY CORRELATED WITH ANALYSES)

POPULATION AND MINERAL ELEMENT	PERCENT MINERAL ABSORBED (DRY WT.)[1] ON LEVELS OF POTASSIUM (PPM.)			
	20	200[2]	500	1000
Nitrogen	2.93	3.16	3.21	3.20
Phosphorus	.261	.076	.185	.082
Potassium	2.00	4.09	6.04	9.07
Calcium	10.99	8.17	5.96	3.93
Magnesium	2.74	1.73	1.33	1.20
Sodium	.217	.255	.259	.388
Iron	.044	.013	.012	.017
Boron	.086	.044	.027	.018
Manganese	.0031	.0019	.0010	.0008
Silicon	.765	.239	.208	.268
Aluminum	.318	.160	.140	.168
Copper	.016	.015	.018	.025
Total Ash	25.3	25.9	27.4	30.6
Mean Mite Population[3]	14.8	17.7	21.8	28.2

[1]Mean of four replicates.
[2]Standard solution level.
[3]Mean progeny of 12 series of 5 female mites each; minimum significant difference between means: 10.3, 1 percent level; 7.9, 5 percent level.

The 100 ppm. nitrogen level constituted a deficiency in the latter stages of tomato plant growth; phosphorus absorption was higher in these plants than in those on the standard solution level. Chapman (1935) and McCalla and Woodford (1938) reported the same phenomenon. Hoagland (1948) stated that it is not unusual for a decrease in absorption of a basic ion to be compensated by an increase in absorption of other bases; he emphasizes, however, that this does not always occur. In this study, all the bases decreased except sodium, which increased slightly at the highest level of nitrogen.

Boron, which appeared to be positively correlated with nitrogen absorption, increased more than 600 per cent over the standard level.

Parks *et al.* (1944), investigating the effects of boron supply on the composition of tomato leaflets, found that nitrogen and phosphorus increased with boron, also that sulfur, magnesium and calcium did not decrease until 0.5 ppm. boron had been supplied in the nutrient solution. Potassium required 15.5 ppm. boron before it decreased.

There seems to be no doubt that nitrogen supply and absorption have a profound influence on other elements. Tests for significance of correlation coefficient demonstrated that five or more of the related elements, as well as total ash, could have been responsible for the

TABLE VI

MEAN MITE POPULATIONS THAT DEVELOPED ON TOMATO PLANTS GROWING IN THREE LEVELS OF CALCIUM SUPPLY, AND MINERAL ANALYSES OF TOMATO FOLIAGE AS CORRELATED WITH POPULATIONS

POPULATION AND MINERAL ELEMENT	PERCENT MINERAL ABSORBED (DRY WT.)[1] ON LEVELS OF CALCIUM (PPM.)			CORRELATION	
	20	200[2]	500	*r* value	Level of significance
Nitrogen	3.19	3.16	3.11		
Phosphorus	.158	.076	.135	+0.585	.05
Potassium	3.58	4.09	4.33		
Calcium	8.69	8.17	10.58		
Magnesium	1.95	1.73	2.05	+0.655	.05
Sodium	1.77	.255	.274		
Iron	.020	.013	.027	+0.635	.05
Boron	.039	.044	.052		
Manganese	.0042	.0019	.0027	+0.777	.01
Silicon	.329	.239	.448	+0.598	.05
Aluminum	.190	.160	.242	+0.598	.05
Copper	.012	.015	.016		
Total Ash	26.2	25.9	28.6		
Total Mite Population[3]	21.8	17.7	15.9		

[1]Mean of four replicates.
[2]Standard solution level.
[3]Mean progeny of 12 series of 5 female mites each; minimum significant difference between means: 7.9, 5 percent level.

population response resulting when nitrogen was supplied at four levels (Table I). The nitrogen-phosphorus relationship and the reaction of boron should be seriously considered as actuators.

Phosphorus

Phosphorus absorption, as evident in this study, was highest at the standard supply level. Negligible amounts of phosphorus were absorbed by the plants at the 5 and the 40 ppm. level, but considerable quantities were absorbed at the 20 ppm. standard solution level (Table II). Nitrogen followed the same pattern to the extent that more nitrogen

was absorbed at the standard solution level than at either of the other two levels.

The other variables agreed with the results of the nitrogen study in that potassium, magnesium and ash attained a low level when nitrogen was highest. The same was true of manganese, copper, iron and aluminum. Accumulating concurrently with nitrogen (as was also the case in the nitrogen study) were phosphorus and silicon. Sodium showed little response to anion variation and calcium maintained an absolute level throughout the three phosphorus levels.

TABLE VII

MEAN MITE POPULATIONS THAT DEVELOPED ON TOMATO PLANTS GROWING IN FOUR LEVELS OF THE STANDARD SOLUTION, AND MINERAL ANALYSES OF TOMATO FOLIAGE; (POPULATIONS NOT SIGNIFICANTLY CORRELATED WITH ANALYSES)

POPULATION AND MINERAL ELEMENT	PERCENT MINERAL ABSORBED (DRY WT.)[1] ON LEVELS OF STANDARD SOLUTION			
	0.2	1.0[2]	2.0	4.0
Nitrogen	2.63	3.16	2.84	3.21
Phosphorus	.629	.076	.139	.175
Potassium	2.52	4.09	4.43	6.00
Calcium	5.79	8.17	9.62	9.11
Magnesium	1.31	1.73	2.58	2.24
Sodium	.303	.255	.202	.191
Iron	.075	.013	.060	.029
Boron	.093	.044	.037	Trace
Manganese	.0043	.0019	.0023	.0013
Silicon	1.122	.239	.888	.446
Aluminum	.351	.160	.412	.257
Copper	.028	.015	.012	.013
Total Ash	19.4	25.9	28.1	30.4
Mean Mite Population[3]	19.8	17.7	39.5	15.4

[1]Mean of four replicates.
[2]Level considered as optimum for growing tomatoes.
[3]Mean progeny of 12 series of 5 female mites each; minimum significant difference between means: 10.3, 1 percent level; 7.9, 5 percent level.

Tests for significance of correlation coefficient produced at least four factors which were apparently responsible for the population changes (Table II). Phosphorus showed a positive correlation, opposite to that demonstrated in the nitrogen study. However, the amounts of phosphorus absorbed covered entirely different ranges in the two studies.

Sulfur

Sulfur absorption was generally concomitant with sulfur supply, although this element decreased slightly in the foliage from the 150 to the 300 ppm. level (Table III). Phosphorus followed a similar pattern and nitrogen was decreased with increasing sulfur supply. Thus there was exerted a competitive effect among the anions. Nightingale

et al. (1933) found that sulfur-deficient tomato plants were high in nitrate.

Calcium and ash (composed largely of calcium, potassium and magnesium) increased considerably with increased sulfur supply; potassium increased slightly. Carolus (1938) found that the sulfate ion had no detrimental effect on calcium absorption in bean plants. Aluminum, silicon and iron were affected, while copper and manganese were not. Boron tended to decrease at the 300 ppm. level. Parks *et al.*, (1944) showed that increased boron depressed the sulfur content of tomato leaflets.

The tests for significance of correlation coefficient demonstrated that at least five minerals were positively correlated with the mite populations that developed on the foliage: magnesium, sodium, potassium, silicon and aluminum (Table III).

Magnesium

Magnesium absorption increased with increased magnesium supply (Table IV). The increased magnesium depressed the other cations, particularly calcium, which was notably decreased. Potassium was depressed slightly while sodium remained constant. Nitrogen was not affected but phosphorus increased with magnesium. The micro-elements exhibited some disturbances in absorption.

Tests for significance of correlation coefficient failed to disclose any significant correlation at the 5 percent level between element absorption and mite population. Phosphorus, however, demonstrated a tendency toward a positive relationship with population.

Potassium

Increased potassium absorption was concomitant with increased potassium supply and this element demonstrated antagonism toward the other cations (Table V). This effect was manifested strongly toward calcium and only slightly less so toward magnesium. Sodium, on the other hand, increased with potassium absorption. Total ash was also increased.

Nitrogen absorption increased gradually with potassium. Phosphorus absorption was highest at the 20 ppm. level, lowest at the standard solution level (200 ppm.) and the 1000 ppm. level. The microelements were affected in diverse ways; boron and manganese were depressed with increased potassium absorption.

Tests for significance of correlation coefficient indicated that these minerals were not correlated to a significant degree with mite population. Potassium and ash produced the highest r values, $+0.443$ and $+0.430$, respectively. Thus, a possible relationship was indicated, even though these figures are short of 0.497 required for significance at the 5 percent level when $n = 16$.

Calcium

Calcium supply was relatively ineffective in increasing calcium absorption (Table VI). Increasing the concentration to 500 ppm. effected a sharp rise in absorption, however, which probably would have continued with higher supply levels, with depression of potassium

and magnesium. It appears that the supply needed to depress those elements is much larger than the unit change of potassium required to depress calcium and magnesium. Increased calcium supply depressed sodium notably.

Ash, magnesium, phosphorus, iron, manganese, silicon and aluminum were absorbed as was calcium. Nitrogen was not disturbed.

Six minerals were significantly and positively correlated with mite populations: magnesium, manganese, silicon, aluminum, iron and phosphorus (Table VI). Calcium and boron showed tendency toward positive correlations but were short of significance at the 5 percent level.

Standard Solution

The general effect of increasing the complete solution concentration was to increase the absorption of macroelements (Table VII). Sodium, however, was depressed as were copper, manganese and boron. Phosphorus, iron, silicon and aluminum were absorbed in the smallest amounts at the 1.0 standard solution level.

The elements apparently were not absorbed in a fashion to be correlated significantly with the mite population.

DISCUSSION OF RESULTS

An increase in mite population seems to occur when the host plant is grown in a medium containing concentrations of salts superfluous for normal plant growth. This fact was borne out in an earlier study (Rodriguez and Neiswander, 1949) which involved 49 commercial greenhouses growing tomatoes, and also in the present report concerning tomatoes grown in nutrient culture. When the standard solution level of elements was doubled in its entire concentration the population developing on those plants was also doubled. It has been demonstrated that insects have distinct tolerances for salt requirements. Bates (1939) showed that the larvae of seven forms of anopheline mosquitoes manifested physiological differences in their toleration for salt solutions. Woodhill (1936) reported that distinct differences in physiological requirements were found between larvae of *Aedes concolor* Tayl. and *Culex fatigans*. The larvae of *A. concolor* required a "balanced solution" of the chlorides of sodium and calcium and would not develop on either salt alone.

As it was the object of this work to seek and identify the more basic factors that encourage or inhibit mite populations, the problem of ionic interrelationships was considered. Foliage analyses disclosed rather definite patterns in ion absorption. There appeared to be, for instance, a general competition among similar ions. Among the cations, potassium, magnesium and calcium depressed the absorption of each other; among the anions, sulfur depressed nitrogen and phosphorus, while the latter two appeared to be absorbed concomitantly. With such relationships existing, it can be seen how certain elements might be identified with a particular type of correlation with mite population in one experiment and with the reverse type in another.

The influence of nitrogen supply on the composition of the foliage was far-reaching. Absorbed nitrogen was negatively correlated with mite population; more than three times as many mites developed on the

plants low in nitrogen (3.27 percent) as on those high in nitrogen (6.11 percent). It appears that the optimum nutritional level of nitrogen for the two-spotted spider mite under the conditions of these tests was not higher than approximately 3 percent dry weight of food.

Nitrogen and phosphorus appeared to be mutually beneficial in assisting the absorption of each other. Phosphorus was absorbed in large quantities when nitrogen supply was increased; because of this wide absorption range, two types of correlations with populations were disclosed. The more common levels of phosphorus content, below 0.30 per cent dry weight of foliage, established a positive correlation. When the phosphorus content exceeded that figure, in the plants grown on 800 ppm. nitrogen, a highly significant negative correlation was demonstrated. Levels of phosphorus similar to the more common levels absorbed by the tomato plant in these studies were utilized by *Tribolium* larvae in the work of Nelson and Palmer (1935); an increase from 0.1 to 0.2 percent phosphorus in the diet enabled the flour beetle to pupate in 35 days less time.

The investigations of McGarr (1942), Evans (1938), and Haseman (1946), mentioned earlier in this report, agreed that the general influence of increased nitrogen on the plant foods of aphids was to effect an increase in progeny. The investigations dealing with the influence of nitrogen on the chinch bug, also mentioned earlier, would indicate that the type of host plant (which may vary in chemical composition) is a determining factor in the development of this insect (Dahms and Fenton, 1940; Dahms, 1947; Haseman, 1946).

A discussion of potassium must of necessity include the other bases, calcium, magnesium and sodium. The interrelationship of these cations must be considered in attempting to associate a population reaction with a given base. The metabolism of nitrogen in the tomato plant was such that increased nitrogen absorption and assimilation caused also an increased uptake of potassium. This in turn effected a decrement in calcium and magnesium uptake and in the percentage composition of these two elements in the plants. Conversely, increased magnesium absorption decreased potassium and calcium absorption. Calcium appeared to be the least mobile of the three in tomatoes, but it displaced sodium considerably and may be able to displace the other bases, given favorable genetic and physico-chemical conditions. The sodium ion apparently was able to replace the potassium ion to some extent. In those cases where an ample supply of potassium was absorbed, however, sodium was generally absorbed concomitantly. Where sodium and potassium were correlated significantly with population, the same type of correlation was demonstrated by both elements. However, the correlation types were reversed in two separate studies. Magnesium established significant positive correlations with population in three different studies. In two of these correlations, there was a corresponding relationship between calcium and population although one test proved slightly short of significance. Calcium and total ash (composed largely of calcium, magnesium and potassium) occurred over a wider range of concentrations in the nitrogen study than in any other study. Both of these variables established significant positive correlations with population in this study.

Although the supply of minor elements was not varied, changes in major element supply effected wide fluctuations in minor element absorption. Boron absorption was affected considerably; this element was associated with nitrogen and phosphorus absorption and was negatively correlated with mite population in the nitrogen study. The concentration patterns demonstrated by manganese in the phosphorus and calcium studies were almost identical but correlations with population were negative and positive, respectively. Silicon and aluminum both were correlated positively with population in the sulfur and calcium studies. Furthermore, silicon absorption followed much the same pattern as phosphorus in most of the studies. Some investigators believe that the presence of this element increases the efficiency of utilization of phosphorus in the plant (Meyer and Anderson, 1946). Iron was found to be positively correlated with population once, in the calcium study, and it is possible that iron may be utilized by the mite. Sharif (1937) found that ferrous ammonium sulfate could be substituted for red cells which ordinarily are included in the diet of yeast and blood serum when rearing larvae of the rat flea, *Nosopsyllus fasciatus* (Bosc.).

As was pointed out earlier in this report, the organic phase of nutrition was not studied, and results that cannot be explained in terms of inorganic nutrition could conceivably be associated with the organic phase of this problem. For example, variations in potassium and standard solution supply were responsible for significant and well-defined changes in mite population, while none of the elements were absorbed in such a way as to be correlated with the resulting population. On the other hand, calcium supply had little effect on the mite population, but six absorbed minerals were found to be positively correlated with population in the calcium study.

SUMMARY AND CONCLUSIONS

1. Studies have been conducted of the effects of supply and absorption of the major elements on populations of the two-spotted spider mite, *Tetranychus bimaculatus* Harvey, developing on tomato plants grown in nutrient culture. The supply of minor elements was not varied.

2. When the concentration of all the major elements was doubled in the nutrient solution, the mite population was likewise doubled.

3. Nitrogen supply was associated with nitrogen absorption which in turn was associated with phosphorus and boron absorption. Nitrogen and boron were negatively correlated with mite population. More than three times as many mites developed on the low nitrogen foliage as on the high nitrogen foliage.

4. Absorbed phosphorus was positively correlated with mite population up to a level of 0.30 percent dry weight of tomato foliage; concentrations above that point were negatively correlated.

5. The absorption of both boron and silicon was associated with phosphorus absorption. Silicon demonstrated positive correlation with population.

6. Although significantly more mites developed in the low sulfur supply level than in the standard level, sulfur absorption failed to show correlation with mite development.

7. There was a general competition in absorption of similar cations. Increased potassium supply and concomitant absorption effected a decrement in magnesium and calcium absorption; increased magnesium supply and absorption caused a decrement in potassium and calcium absorption.

8. Sodium and potassium were generally absorbed concomitantly. Potassium supply was positively related to mite development, but absorbed potassium and sodium were, in different studies, both positively and negatively correlated with mite population.

9. Magnesium supply showed indications of a positive relationship with mite development and magnesium absorption was positively correlated with population in three studies. Absorbed calcium produced a positive correlation with population in the nitrogen study.

10. Variations in major element supply effected wide fluctuations in the absorption of minor elements, which exhibited some significant relationships with populations. The percentage total ash was positively correlated with population when the nitrogen supply was varied.

11. These studies showed that foliage analysis can point out relationships not otherwise evident between phytophagous pests and nutrients in mineral nutrition investigations. It was recognized that some results could not be explained solely on the basis of inorganic nutrition.

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CONTRIBUTIONS TO THE METAMORPHOSIS AND BIOLOGY OF THE GENUS ARTICHOPOGON KIEFER (DIPTERA, CERATOPOGONIDAE) WITH REMARKS ON THE EVOLUTION AND TAXONOMY OF THE GENUS, by ANKER NIELSEN. 99 pages, 2 plates, 33 text figures. Det Kongelige Danske Videnskabernes Selskab Biologiske Skrifter, Bind vi, nr. 6, Copenhagen. Price, $2.75.

This intensive study of eight aquatic species, six of which were reared to adults and described as new, is a contribution to our knowledge of a heleid genus which was previously poorly known in its immature stages. The author concludes that the immature stages furnish much better characters for taxonomic differentiation than do the adults.—M. T. J.

INTRATRACHEAL PRESSURE IN INSECT RESPIRATION

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Most adult insects maintain a direct gaseous exchange between the environment and tissues by means of a tracheal respiratory system. Packard (1898), Vinal (1919), Snodgrass (1925, 1935) and Wigglesworth (1939) have extensively investigated and described the morphology of the insect respiratory system. Du Buisson (1924), Lee (1925, 1927), McKay (1927), Uvarov (1928), McArthur (1929), McGovran (1931) and Kitchel and Hoskins (1935) have investigated the coordination of abdominal movement, opening and closing of spiracles and the passage of air through the tracheal systems of several orthoptera. These investigations established the concept that there is a directed tracheal ventilation in insects. Inspiration occurs through an anterior group of spiracles. The air is then passed through the tracheal system and expired through a posterior group of spiracles. However some of these workers found that occasionally any of the spiracles might serve for both inspiration and expiration and that when the grasshopper was struggling or respiration was stimulated by carbon dioxide the normal direction of tracheal ventilation might be reversed. In all cases it was thought that the respiratory cycle consisted only of inspiration and expiration which ventilated the larger trachea and air sacs. Oxygen was then carried through the smaller trachea and air sacs by diffusion to the site of absorption at the end of the tracheoles. The tracheal system was considered to be open to the air at all times and in no case was the intratracheal pressure believed to be greater than ambient pressure.

McCutcheon (1940) demonstrated for the first time the existence and function of a positive pressure in the tracheal system of an insect. He mechanically recorded the abdominal respiratory movement and the opening and closing of selected spiracles of the grasshopper *Dissosteria carolina* Linn. and found that each respiratory cycle consisted of an inspiratory, compressatory and expiratory phase. During the compressatory phase he showed that all spiracles were closed during abdominal contraction. Thus pressure was exerted on the air trapped in the tracheal system. McCutcheon described two distinct types of respiratory cycles. The normal cycle occurred while the grasshopper was at rest and could be distinguished from a forced or dyspneic cycle produced by mechanical irritation, heat or ether. By sealing a capillary manometer over the second thoracic spiracle he estimated a maximum intratracheal pressure of 2 mm. Hg during the normal cycle and 7 mm.

[1]This work was done in the Zoology Laboratories at Duke University. I wish to express my appreciation to Dr. F. G. Hall for his many helpful suggestions during the progress of this work.

Hg during the dyspneic cycle. By direct observation McCutcheon found the smaller air sacs more distal from the spiracles were more fully inflated during the dyspneic than during the normal respiratory cycle. By means of an ingenious mechanical model he was able to show how increased intratracheal pressure could force air along the tracheal system from the larger to the smaller air sacs.

In the present investigation a method has been devised for simultaneously recording intratracheal pressure, abdominal and spiracular movement. When recording only intratracheal pressure the insects could be left free in a chamber to assume a normal position or to move at random. The relationship of abdominal and spiracular movement to intratracheal pressure has been thoroughly investigated in the grasshopper. Conditions affecting intratracheal pressure in grasshoppers have been studied in order to establish the physiological significance of this pressure. By use of this improved apparatus it has been possible to demonstrate the existence of a functional intratracheal pressure in a large number of insects.

METHODS

If an insect is placed in a closed chamber the pressure in the chamber will drop in proportion to the positive pressure exerted on the gas in the tracheal system of the insect during each respiratory cycle. By recording the pressure changes in the chamber an indirect recording of the intratracheal pressure would be obtained. Hurst (1941) has developed a recording differential optical manometer that is sufficiently sensitive to record this change of pressure when selected insects respire in closed containers with volumes in the range of 5 to 500 ml.

The construction and operational characteristics of the manometer used in these experiments are the same as those described by Hurst (1941). The manometer consists of two closed compartments separated by a very sensitive rubber membrane which activates a small mirror when a pressure differential occurs between the two chambers. A light beam reflected from the mirror records this movement on a suitable photographic recorder. To make a recording the inner manometer compartment was connected to the chamber containing the insect. The outer compartment remained closed to eliminate the effect of ambient atmospheric pressure changes. The sensitivity of the manometer could be varied by adjusting the suspension of the mirror. The sensitivities used in these experiments ranged from 0.00055 to 0.00150 mm. Hg per mm. deflection of the light beam on 6 cm. recording paper at a distance of one meter. The arrangement of the apparatus for recording intratracheal pressure and abdominal movement is shown schematically in figure 1. The insect was fastened at the sternum by means of beeswax on a support in the respiration chamber J. Two sides of this chamber were made of optical glass. A standard lantern projector, indicated by the lens K was used to project the dorsal and ventral surface of the abdomen on the recording camera F. A hair line of light formed by slit D was focused on the manometer B and reflected to the camera F to record intratracheal pressure in the shadow cast by the insect's abdomen. The intensity of the source lights E and I was controlled by the variable resistors G. Since the assembly

was extremely sensitive to temperature changes a screw clamp was used on the tube connecting the respiration chamber to the manometer to zero the pressure base. In another series of experiments in order to record the opening and closing of spiracles it was necessary to remove the projection lantern so two observers could focus binocular microscopes on the spiracles through the optical wall of the respiration chamber. The slit D was replaced by a filament so the entire width of the photographic recording paper was exposed with the intratracheal pressure curve showing as a white line on the exposed paper. The two observers operated keys which activated signal markers recording along the margin of the film to show opening and closing of two pair of spiracles. In experiments in which the effect of temperature and gas mixtures on

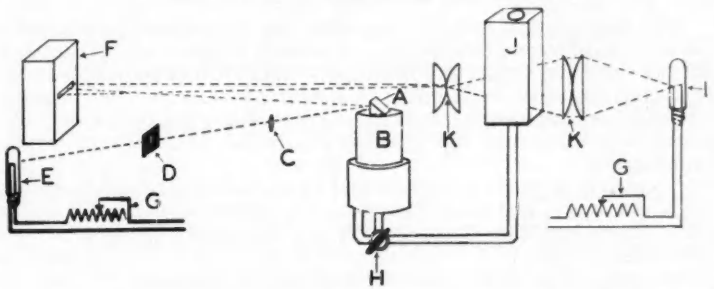

FIG. 1. Diagrammatic drawing showing arrangement of apparatus used for simultaneously recording intratracheal pressure and abdominal movement of insects. See text for explanation.

respiration was recorded over a period of time the entire manometer assembly and respiration chamber was immersed in a water bath to the level of the top of the manometer. All recordings were made on 6 cm. contrast photographic recording paper. In order to obtain better reproductions exact tracings of these photographic records have been used in this publication.

De Moll's (1927) method was used to estimate the volume of the tracheal system of insects in order that the intratracheal pressure produced during the compressatory phase of the respiratory cycle could be calculated. Immediately after inspiration, when the tracheal system was judged to be most completely filled, the grasshopper was thrust beneath a water filled funnel with a calibrated side arm. The gas was liberated from the tracheal system by dissection and maceration, collected over water and the volume immediately determined. It is obvious that this method gives only an approximation of the tracheal volume. Errors are introduced by bubbles clinging to the insect, absorption of carbon dioxide by the water, failure to liberate all gas from the tracheal system and most of all by the extent to which the tracheal system is filled when the insect is submerged. Assuming the vital capacity of these grasshoppers is between twenty and forty percent of the tracheal volume (Wigglesworth, 1939; Krogh, 1941) it is reasonable to believe the method is at least within this range of accuracy.

Once the volume of the tracheal system had been estimated and the volume of the respiratory chambers was known the intratracheal pressure exerted during the compressatory phase was calculated as follows:

$P V = P' V'$ where
P = Intratracheal pressure,
V = Volume of gas in insect tracheal system,
P' = Maximum pressure in respiration chamber during respiratory cycle,
V' = Volume of respiration chamber and connecting system less volume of insect.

RESULTS

Respiratory mechanisms in grasshoppers

The mechanics of external respiration was most extensively investigated in grasshoppers because they were easily obtained and of excellent size for recording respiratory movement, intratracheal pressure and for observing spiracular movement. *Schistocerca obscura* was most readily available and was used in most experiments. For comparative purposes *Schistocerca americana* and *Dissosteria carolina* were used in several experiments.

Typical respiratory movement and intratracheal pressure recordings for *S. obscura* are shown and described in figure 2. Recordings A and D show the relationship of the opening and closing of the spiracles, and intratracheal pressure. Previous observations had shown that in *S. obscura*, as in other grasshoppers, the first four pair of spiracles operated together and normally served for inspiration. The last six pair of spiracles opened and closed simultaneously and normally served for expiration. Thus the opening and closing of the second and tenth pair of spiracles is representative of the movement of the two groups of spiracles. Abdominal respiratory movement and pressure were recorded simultaneously in records B, C and E were superimposed from a study of many records of the type shown in A and D on which the opening and closing of the spiracles had been recorded.

S. obscura exhibits three distinct types of respiratory movements. The cycle which occurs most frequently when the insect is at rest and which we shall refer to as the normal cycle is shown in records D and E, figure 2. Inspiration occurs through the anterior spiracles as the abdomen expands and the intratracheal pressure drops to zero, the posterior spiracles being in the closed position. During the compressatory phase the anterior spiracles close, the abdomen contracts, the posterior spiracles remain closed and the intratracheal pressure rises. During expiration abdominal contraction continues, and expiration occurs through the posterior spiracles which open at the beginning of this phase. The anterior spiracles remain closed. The intratracheal pressure drops sharply from the maximum at the instant the expiratory spiracles open. Thus in this normal respiratory cycle air is drawn in through the anterior group of spiracles, compressed and passed through the tracheal system to be expired through the posterior spiracles. This cycle for *S. obscura*, based on the correlation of intratracheal pressure, respiratory and spiracular movement is identical to that derived by McCutcheon (1940) for *D. carolina* from mechanically

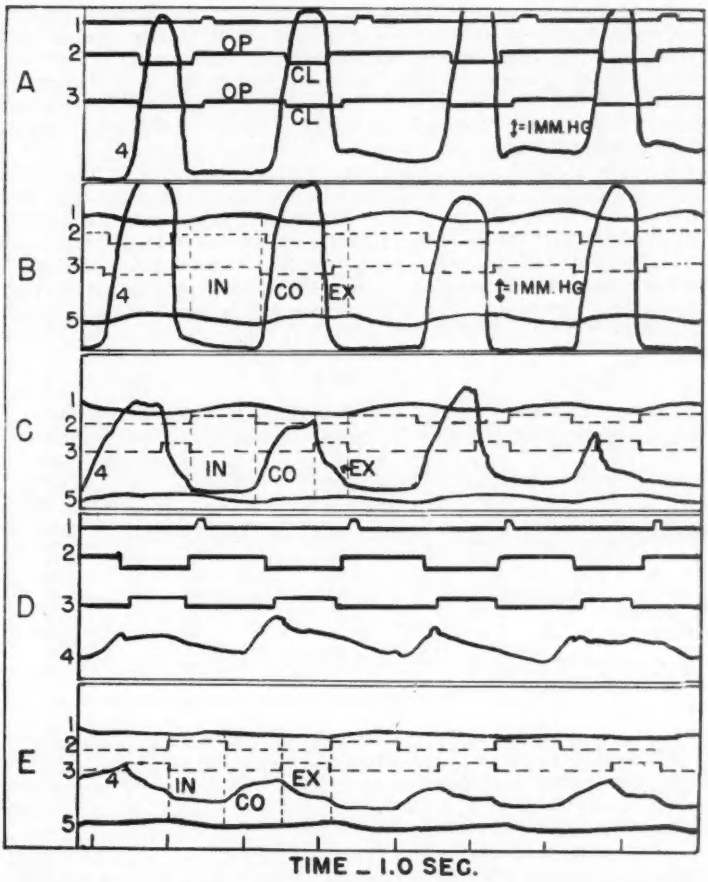

FIG. 2. Records showing normal and dyspneic respiratory movements of the grasshopper, *Schistocera obscura*. Records A and D made with one grasshopper; records B, C and E made with a second grasshopper. The intratracheal pressure scales for the two grasshoppers shown on records A and B were calculated by the method described in the text. Time is shown at the bottom of the plate and is the same for all records.

Legends for A and D. 1. Beginning of respiration indicated by upward deflection of signal marker. There is probably a significant lag in this measurement since the exact beginning of the inspiratory movement was difficult to observe. 2. Second thoracic spiracles: up, open; down, closed. 3. Tenth pair of spiracles: up, open; down, closed. 4. Intratracheal pressure.

Legends for records B, C and E. 1. Movement of tergum of fourth abdominal segment. 2. Schematic position of second pair of abdominal spiracles: up, open; down, closed. 3. Schematic position of tenth pair of spiracles: up, open; down, closed. 4. Intratracheal pressure. 5. Movement of sternum of fourth abdominal segment.

recording respiratory and spiracular movement. A different type of movement is shown in record C and is the same as the dyspneic or forced cycle described by McCutcheon (1940). This cycle is the same as the normal cycle except that abdominal contraction is deeper and a much higher intratracheal pressure is produced during the compressatory phase. Gas is passed through the tracheal system in the same manner as in the normal cycle.

Records A and B show a third type of respiratory cycle in *S. obscura*, dyspneic in nature, in which all spiracles serve for both inspiration and expiration. This cycle may also be divided into inspiratory, compressatory and expiratory phases. Inspiration occurs during abdominal expansion with all spiracles open. At the beginning of abdominal contraction all spiracles close and intratracheal pressure rises very rapidly to a maximum. The anterior group of spiracles always opened momentarily before the posterior group. Thus it is possible that during

TABLE I

INTRATRACHEAL PRESSURES RECORDED IN ADULT FEMALE GRASSHOPPERS

Species	Weight—Grams	Volume of Tracheal System—CC.	Intratracheal Pressure	
			Normal MM. HG.	Dyspneic MM. HG.
S. americana	1.28	0.64	0.86	9.67
"	2.34	0.98	0.73	9.41
"	2.17	0.93	0.90	9.34
"	2.10	0.73	0.91	7.47
S. obscura	3.16	0.96	0.71	7.58
"	2.01	0.74	0.59	6.60
"	2.70	0.95	0.74	8.90
"	2.93	0.88	0.75	9.10

this respiratory cycle most of the expired gas escapes through the anterior spiracles which would be in agreement with the findings of McKay (1927), McArthur (1929) and McGovran (1931). This type of respiratory movement in which all spiracles served for both inspiration and expiration occurred very frequently in *S. obscura*, but less often if at all in *S. americana* and *D. carolina*. The shape of some of the intratracheal pressure curves for these latter species indicated this type of cycle was occurring but it appeared with such infrequency that actual records correlating spiracular movement with intratracheal pressure were never obtained.

Estimation of Intratracheal Pressure in Grasshoppers

Table I shows intratracheal pressure estimations for two species of grasshoppers. These values were calculated from records made over a period of three months, using respiration chambers of various volumes and several manometer sensitivities. The values are averages for 10 consecutive respiratory movements of the normal or dyspneic type. By mechanically varying the pressure in the respiration chamber it was found that manometer recordings with an accuracy of 2 percent could

be maintained. The greatest error in these calculated pressures is then introduced by the error in the determination in tracheal volume. However in comparative experiments when the same insect was used in

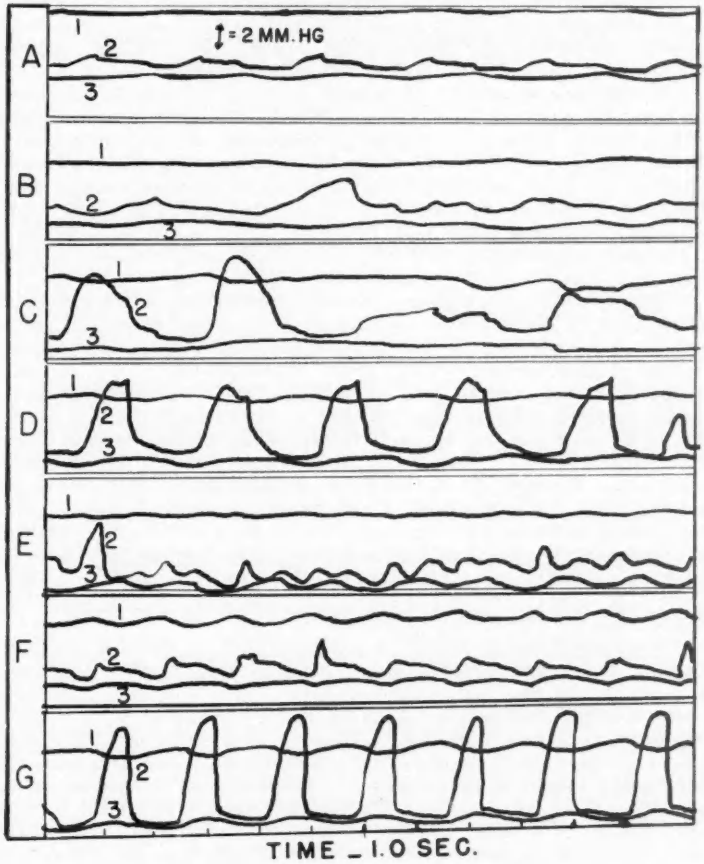

FIG. 3. Typical records showing the effect of activity, increased CO_2, high temperature, and decreased O_2 on intratracheal pressure and respiratory movement in the grasshopper. An intratracheal pressure scale, calculated by the method described in the text, is shown on record A. All records were made with a single grasshopper in the same chamber so the height of the intratracheal pressure curve shows relative intratracheal pressure for each condition. The temperature was 27° C. for all experiments except E. Adult female *Schistocera obscura*, weight 2.93 grams. A and B. Normal cycle while resting. C. Dyspneic breathing while struggling. D. Dyspneic respiration immediately after struggling. E. Dyspneic respiration produced by high temperature (40° C.). Note deep abdominal movement without increased intratracheal pressure. F. Dyspneic breathing in 10 per cent CO_2. G. Dyspneic breathing in 8.3 per cent O_2.

the same chamber the height of the pressure curve is believed to be proportional to intratracheal pressure within the 2 percent accuracy of the instrument provided the tracheal system is filled with gas to the same degree during each compression.

Effect of Temperature, Carbon Dioxide and Oxygen on Intratracheal Pressure in Grasshoppers

The normal respiratory movement with low intratracheal pressure and the dyspneic type with high pressure are definitely correlated with the metabolic requirements of the grasshopper. During active movement and immediately following activity the dyspneic cycle predominates. After periods of 2 to 3 hours rest in the respiration chamber the normal type only would occur for 15 to 30 minute intervals. Representative recordings shown in figure 3 show the effect of activity, decreased oxygen, and increased carbon dioxide and increased temperature on respiration of the grasshopper.

Three experiments were conducted in which ten minute recordings were made of intratracheal pressure at 5° C. increments from 15° to 40° C. Except for an increase in respiratory rate there is no significant change in respiration from 15° to 30° C., both normal and dyspneic cycles appearing as usual. At 35° C. all respiratory movements appear to be deep and forced. The pressure curves characteristic of normal and dyspneic cycles still appear. However the pressure exerted during the dyspneic cycle is no longer as high as that observed under normal conditions. Record E, figure 3 shows abdominal movement and intratracheal pressure at 40° C. compared to that at 27° C. The ventilating movement is very deep but the high intratracheal pressure characteristic of dyspneic movement at lower temperatures has disappeared entirely. By direct observation it was found that the spiracles were open during most of the cycle and clicked shut only momentarily toward the end of abdominal contraction.

Grasshoppers were placed in concentrations of 1%, 5% and 10% carbon dioxide and observed as in previous experiments. Increasing the carbon dioxide increased respiratory rate and anplitude with the usual normal dyspneic cycles occurring at concentrations of 1% and 5%. However as shown in record F, figure 3 at a concentration of 10% carbon dioxide the respiratory movements are very deep but the intratracheal pressure characteristic of forced breathing under normal conditions no longer occurs. Direct observation showed the anterior and posterior spiracles alternately opened and closed. The slight, sharp increase in intratracheal pressure occurred at the instant the posterior spiracles closed.

By reducing the partial pressure of oxygen in the inspired air it was found that dyspneic respiration with very high intratracheal pressure could be initiated and maintained by the grasshopper. A typical experiment is shown in record G, figure 3. In an atmosphere containing 8.3% oxygen this type of respiration was observed to continue for an hour or more with no indications of impairment to the insect. The average of results from experiments with three *S. obscura* showed that in 8.3% oxygen the respiratory rate increased 82% and the intratracheal pressure increased 66% over the normal value obtained in atmospheric air.

Intratracheal Pressure in Insects other than the Grasshopper

A series of recordings were made to determine whether or not representative species of several orders of insects produced a significant intratracheal pressure during respiratory movement. Insect chambers and manometer sensitivities were selected to produce a readily recordable deflection of the light beam during each respiratory movement. Figure 4 shows respiratory movement and intratracheal pressure in

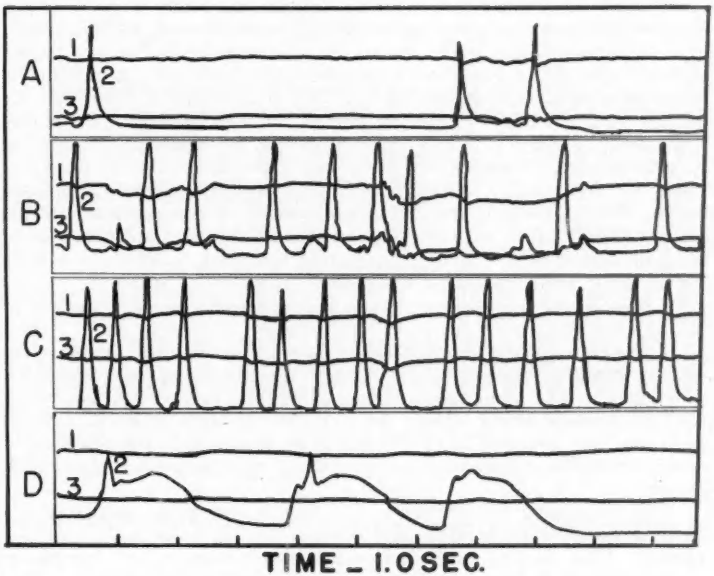

FIG. 4. Normal types of respiratory movement and intratracheal pressure curves recorded for the cockroach, *Periplaneta americana*. All records were made from a single insect in the same respiration chamber so intratracheal pressure is proportional to the height of the pressure curve for the different records. No quantitative estimation of intratracheal pressure could be made because the tracheal volume was not determined. Legend.—1. Movement of tergum of fourth abdominal segment. 2. Intratracheal pressure. 3. Movement of sternum of fourth abdominal segment.

the cockroach *Periplaneta americana*. Respiratory movement is rather erratic. During quiescence no movement is observed for periods of a minute or more. During and immediately after activity very rapid ventilating movements would appear. Record A, figure 4 was made while the cockroach was quiet, record B while struggling and record C immediately after activity. These records show a definite intratracheal pressure is produced by each twitch-like contraction of the abdomen. Direct observation under the binocular microscope showed that thoracic spiracles closed with each of these movements

but the rapidity of the contraction prohibited a correlation of spiracular movement with intratracheal pressure. Record D, figure 4 shows a distinctly different type of movement which occurred less frequently in the cockroach. This is more like the pumping type of respiratory movement observed in the grasshopper.

Figure 5 shows intratracheal pressure curves for several insects. Records A, B, C and D are all for insects which fly and show a very rapid respiratory movement. Records E and F are of interest in that a very slow respiratory rate is shown for the non-flying beetle *Passalus cornutus* whereas the more active flying beetle *Cotinis nitida* shows a pattern very similar to the dragon fly. Records G and H are typical pressure curves for long-horned grasshoppers and are quite different from those of acridid grasshoppers. Records I and J show that the normal and dyspneic pressure curves in the acridid grasshopper *Dissosteria carolina* are essentially the same as those for the acridids *S. obscura* and *S. americana* previously described. An attempt was made to demonstrate intratracheal pressure in a large cerambycid beetle larva. Though the larva was placed in the smallest possible respiration chamber with the manometer at the maximum sensitivity no deflection of the recording beam was observed. This indicates the beetle larva never created a significant intratracheal pressure.

DISCUSSION

These experiments have definitely shown that all adult, active insects studied compress the gas in the tracheal system during each respiratory movement. The fact that increased activity and decreased oxygen tension always bring about an increase in intratracheal pressure indicates this pressure is of physiological importance for transporting oxygen from the spiracles to the more distal ramifications of the tracheal system.

The extensive investigation of the respiratory mechanisms in *Schistocerca obscura* confirm and expand the findings of McCutcheon (1940). The normal and dyspneic cycles consisting of inspiratory, compressatory and expiratory phases described by McCutcheon (1940) for *D. carolina* were observed in *S. obscura*. The dyspneic cycle did not necessarily produce a high intratracheal pressure. If the dyspneic respiration was initiated by activity or low oxygen tension the intratracheal pressure was greatly increased. However, violent respiratory movement resulting from increased carbon dioxide or high temperature did not increase the pressure in the tracheal system above normal values. McCutcheon (1940) could not make these distinctions in the dyspneic cycle of the intratracheal pressure with the apparatus used at that time.

The data on tracheal ventilation presented in the present paper are consistent with the idea that oxygen is primarily absorbed through the tracheoles whereas carbon dioxide diffuses through the tracheoles, air sacs, trachea and even the external cuticle (Wigglesworth, 1939). It is suggested that the dyspneic cycle with the high intratracheal pressure initiated by increased metabolic demands or low oxygen tensions is an adaptation for forcing oxygen along the tracheal system toward the site of absorption. The forced respiratory cycle resulting from increased

carbon dioxide or increased temperature probably passes large quantities of air in and out of the proximal portions of the tracheal system. This should be quite effective for removing carbon dioxide or evaporating water to lower the body temperature. Whether or not these mechanisms are actually adapted for these purposes will have to be determined by more critical experiments.

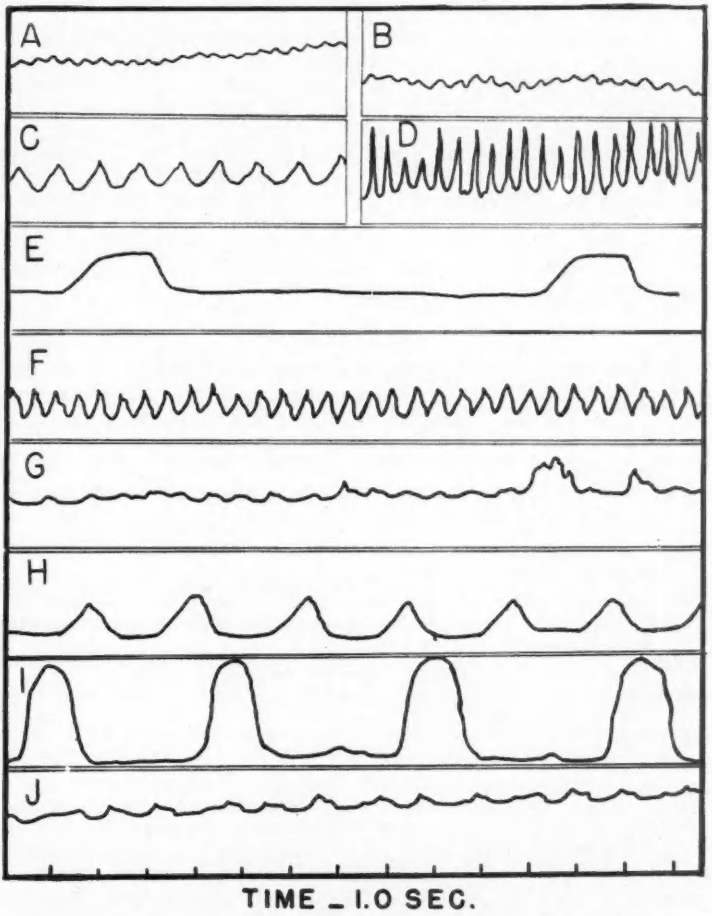

FIG. 5. Records showing typical intratracheal pressure curves for several representative insects. A. Bumble bee, *Bombus pennsylvanicus*. B. Wasp, *Polistes annularis*. C. Dragon fly, *Pachydiplax longipennis*. D. Tabanid fly, *Tabanus* sp. E. Beetle, *Passalus cornutus*. F. Beetle, *Cotinis nitida*. G. Long-horned grasshopper. H. Long-horned grasshopper, *Scudderia* sp. I. Acridid grasshopper, *Dissosteria carolina*, after activity. J. *Dissosteria carolina*, resting.

SUMMARY

A new and improved method for recording intratracheal pressure in insects has been developed, described, and used. A very sensitive differential manometer was used to indirectly record the intratracheal pressure in representative insects.

All adults, active insects studied have a compressatory phase in each respiratory movement. A significant pressure is exerted on the gas trapped in the tracheal system.

The mechanism of respiration was extensively investigated in the grasshopper *S. obscura*. Three distinct types of respiratory cycles were observed and described. Each cycle consisted of an inspiratory, compressatory and expiratory phase. The first type of respiratory cycle, described as the normal cycle, occurred most often during rest with an intratracheal pressure between 0.6 and 1.0 mm. Hg. The second was a forced or dyspneic respiratory cycle occurring during activity or initiated by low oxygen pressures, in which respiratory movement was rapid and deep with an intratracheal pressure in the range of 7–10 mm. Hg. A third type of movement, induced by high carbon dioxide tension and by high temperatures was rapid and deep but the intratracheal pressure was in the range of 1 to 2 mm. Hg.

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NORMAL TOTAL HEMOCYTE COUNTS OF TENEBRIO MOLITOR[1]

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Tauber and Yeager have published total hemocyte counts of 739 individuals from 82 species in the orders Orthoptera, Odonata, Hemiptera, Homoptera (1935); and in Neuroptera, Coleoptera, Lepidoptera, and Hymenoptera (1936). Included were counts of 104 insects from 19 species of Coleoptera; the lowest normal average count was 2,889 cells/mm^3 (*Lixus mucidus* Lec.), and the highest normal average count was 68,328 cells/mm^3 (*Galerita janus* Fab.). The pooled mean for the normal Coleoptera examined by these workers was 27,669 cells/mm^3. Prior to these studies the same authors (1934) made 220 counts from larger nymphs and adults of the field cricket, *Gryllus assimilis pennsylvannicus* Burm. In this insect the normal total hemocyte count ranged from 15,000 to 275,000 cells/mm^3, with an average of 70,118 cells/mm^3.

The present paper reports 275 total hemocyte counts together with analysis of variance[3] from larvae of the common yellow mealworm, *Tenebrio molitor* L. (Coleoptera). Some extra attention is given to the statistical measure of these counts inasmuch as they will be the basis of comparison for other counts after larvae are subjected to various experimental procedures.

ANIMALS

Tenebrio molitor larvae were selected from stock cultures maintained at 25° to 30° C., and fed whole wheat bran, corn meal, and commercial dog food. Only large larvae, measuring 20 mm. to 30 mm. in length, were used because they were more conveniently manipulated and gave larger quantities of hemolymph, thus facilitating the determination of both total and differential hemocyte counts from the same insect.

The larvae were considered normal, although all examined contained gregarines. Summer (1933, 1936) has indicated that these protozoa are important in the health, size, and longevity of the mealworm.

METHODS

Twenty-five larvae were used for each of the eleven length categories. Care was taken not to overmanipulate the insect prior to fixation, and all counts were made in the afternoons to reduce the possibility of diurnal variations. Larvae were individually immersed in water at a

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constant temperature of 55° C. for 2 minutes (to fix the hemolymph cells), removed, thoroughly dried, and measured. Immediately thereafter the anterior pair of larval legs was cut off and a large drop of hemolymph placed onto a microscope slide previously coated with a thin layer of paraffin.

A Thoma white blood cell pipette was used to draw this heat-fixed hemolymph to the 0.1 mark. The pipette was then quickly filled to the 11 mark with the diluting fluid recommended by Tauber and Yeager (1935). The standard technique for white counts as used for vertebrate leucocytes was employed (Beck, 1938). Individual counts were made by averaging a 4 square count from one chamber with a check count from the other chamber of the Neubauer slide. If the check count differed by more than 10,000 the entire sample was discarded. This practice may have introduced an error because a difference of 20,000 would have been possible when the total hemocyte count was high. Consequently, this procedure may have tended to underestimate the mean counts for all length classes, and the extent of underestimation would be greatest for those with high means. However, this underestimation does not invalidate significant differences in the counts reported here.

RESULTS

Total hemocyte counts for 275 mealworm larvae are summarized in Table I, with analysis of variance shown in Table II.

Different length classes have different means and these differences seem to be accounted for principally by linear regression between counts and length. Statistical treatments show that as length increases the T.H.C. decreases. The higher T.H.C. in the smaller length classes is highly significant. While the individual total hemocyte count values from the larvae examined ranged from 9,200 to 128,000 cells/mm,^{3} 88.2% ranged from 20,001 to 70,000; 76.9% ranged from 20,001 to 60,000; and 65.3% ranged from 30,001 to 60,000. The + and − differences of the group means as compared to the pooled mean were 5,259 and 9,393, respectively. Standard deviation (including error of mean of two counts) based on pooled mean square within length classes is 17,000. The standard error of mean shown, based on 25 insects, is 3,400.

Apparent hemocytosis, occurring in 8.4% of normal larvae examined, may be defined as a total hemocyte count value in excess of 90 percentile of all values. For normal distribution this is the mean + 1.28 standard deviations. For a standard deviation of 17,000 this would be a value in excess of the mean + 22,000. Taking into account the regression of count on insect length, this would be a value in excess of 1,000 (95 − length in mm.).

Apparent hemocytopenia, occurring in 2.9% of normal larvae examined, may be defined as the total hemocyte count value below the fifth percentile of all values. For a normal distribution this is the mean − 1.64 standard deviations. For a standard deviation of 17,000 this would be a value below mean − 28,000. Taking into account regression of count on insect length this value would be below 1,000 (45 − length in mm.). Obviously this applies only in the 20 to 30 mm. length range.

TABLE I

TOTAL HEMOCYTE COUNTS (T.H.C.) FOR 275 MEALWORM LARVAE, 25 IN EACH LENGTH CATEGORY

Length mm.	Mean T.H.C. Cells/mm³	Total Range
20	56,820	20,500 — 128,000
21	57,036	25,000 — 83,000
22	44,712	14,200 — 77,500
23	45,400	17,000 — 92,000
24	47,312	19,000 — 89,000
25	47,400	15,200 — 82,500
26	47,800	22,500 — 70,700
27	42,384	20,500 — 79,500
28	45,236	16,500 — 105,000
29	46,472	9,200 — 88,000
30	43,500	20,700 — 90,700
	47,643	

TABLE II

ANALYSIS OF VARIANCE OF TOTAL HEMOCYTE COUNTS

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F	Significance
Between length classes	$6{,}068 \times 10^6$	10	607×10^6	2.08	$P < 0.05$
Linear regression against length	$2{,}958 \times 10^6$	1	$2{,}958 \times 10^6$	10.12	$P < 0.005$
Deviations from regression	$3{,}110 \times 10^6$	9	346×10^6	1.18	Not significant
Within length classes	$77{,}149 \times 10^6$	264	292×10^6		
Total	$86{,}193 \times 10^6$	274			

TABLE III

CRITICAL VALUES OF HEMOCYTOSIS AND HEMOCYTOPENIA IN MEALWORM LARVAE

Length mm.	Critical Values Hemocytosis x 10^3	No. Counts Hemocytosis	Critical Values Hemocytopenia x 10^3	No. Counts Hemocytopenia
20	75	4	25	1
21	74	5	24	0
22	73	1	23	2
23	72	2	22	1
24	71	3	21	1
25	70	1	20	1
26	69	1	19	0
27	68	1	18	0
28	67	2	17	1
29	66	1	16	1
30	65	2	15	0

DISCUSSION

A number of possibilities have been suggested to account for variations in total hemocyte counts of insects, including (1) temporary cessation of hemocyte flow, (2) amount and type of diet, (3) state of nutrition, and (4) inadvertent coagulation at time of collecting hemolymph samples for counts (Tauber and Yeager, 1934). Hemocytosis, comparable to leucocytosis in the vertebrate, has been reported for a number of insect orders by Tauber and Yeager (1935, 1936) who consider it to accompany certain physiological and pathological conditions such as ecdysis, oviposition, and parasitism (1934). Hemocytopenia, comparable to a leucopenia in the vertebrate, appears to be associated with acute bacterial infections (Babers, 1938) and with certain kinds of poisons (Fischer, 1936). Hemocytopenia occurs normally at pupation (Tauber and Yeager, 1936).

In spite of the wide variations in the hemocyte counts of the mealworm the mean counts are reproducible. The younger, smaller mealworm larvae have higher total counts than older larvae which are approaching pupation. The adults of holometabolous insects have been shown to have lower counts than larvae (Tauber and Yeager, 1936). While Tauber and Yeager (1936) suggested that holometabolous insects may develop a new set of imaginal hemocytes, mitotic indices from stained smears and cross sections during the pupal and early adult stages clearly indicate that this does not take place in the mealworm. Nor does the suggestion that holometabolous insects develop or possess different types of hemocytes in the adult stage apply to the mealworm (Jones, 1950), although it does apply to the southern armyworm (*Prodenia eridania*) as shown by Yeager (1945).

Since preliminary observations by the present writers indicate that *Tenebrio* larvae do not normally possess hemocytopoietic organs comparable to those in some other Arthropoda, hemocytosis in the mealworm is believed to be brought about either by mitotic division of hemocytes in the circulating hemolymph, or by a flushing out of hemocytes from some fortuitous temporary site of accumulation in the hemocoel, or both. Hemocytopenia in the mealworm appears to be due to a nonspecific adhesion to tissues.

Neither diurnal variation, seasonal variation, or variations due to stress occurred with the present species.

SUMMARY AND CONCLUSIONS

(1) Average total hemocyte counts for 275 *Tenebrio molitor* larvae, measuring 20 to 30 mm. in length, are presented together with analysis of variance.

(2) Different length classes have different mean total hemocyte counts and these differences are accounted for by linear regression between counts and length. Younger, smaller larvae have significantly higher total counts (approximately 57,000 per mm^3) than older larvae (approximately 44,000 per mm^3) approaching pupation.

(3) Apparent hemocytosis occurred in 8.4% of normal larvae, and is considered to result from mitotic division of circulating hemocytes or by release of hemocytes from some temporary accidental site of accumulation, or both.

(4) Apparent hemocytopenia occurred in 2.9% of normal larvae and is considered to be due to nonspecific adhesion of hemocytes to tissues.

(5) The mean total hemocyte count of 275 mealworm larvae is 47,643 cells/mm^3 with a standard deviation of 17,000 and standard error of means, based on 25 insects, 3,400.

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A FIELD GUIDE TO THE BUTTERFLIES OF NORTH AMERICA, EAST OF THE GREAT PLAINS, by ALEXANDER B. KLOTS. xvi+349 pages, 40 plates. Houghton Mifflin Company, Boston, 1951. Price, $3.75.

This book, the first of the Peterson Field Guide Series to deal with an entomological subject, is an addition to the literature of lepidopterology which will be welcomed by the amateur and professional worker alike. Actually it is much more than a field book. It embodies the author's interpretation of modern butterfly taxonomy, based on the dynamic concept of the species; in addition it contains a considerable amount of ecological and life-history material. The approach is made from the standpoint of field recognition, coupled with the necessity of collecting material and making identifications from cabinet specimens. For identification purposes, wing patterns and coloration furnish the most important set of characters. The brief diagnoses of the species call attention to characters of importance for recognition, and differentiations are clearly made between similarly appearing species that may occur in one range. Keys are sometimes, though not universally employed. A unique feature of the illustrations, and one that should aid greatly in ready identification, is the system of indicating diagnostic pattern and color peculiarities by means of guide lines. The illustrations, both black-and-white photographs and color drawings, are copious and well executed and, for the most part, well reproduced.

Dr. Klots' book mirrors the personality of the author, a man to whom each field trip is an interesting experience, to be enjoyed as such and not for the harvest in specimens which it will reap. His point of view consequently gives his book a freshness such as is not found in most taxonomic works. From the standpoint of a western entomologist, we regret that the geographical scope covers only North America *east* of the Rockies.—M. T. J.

THE BIONOMICS OF DERMESTES MACULATUS DEG.

II. Larval and Pupal Development at Different Moisture Levels and on Various Media[1]

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The number of larval stages and times required for larval and pupal development of *Dermestes maculatus* Degeer (Coleoptera, Dermestidae) have been noted in several instances. In most cases, however, rearing conditions, such as temperature, humidity, nutrition, etc., are not clearly defined, or are not stated at all. Sometimes only a very few individuals made up the test population sample. Consequently, because life history observations were carried out under such varying environmental conditions, reports of number of instars and time spent in instars have not been found in agreement. As with other dermestids, larvae of *D. maculatus*, even when reared under regulated laboratory conditions, may have an inconstant number of instars. Therefore, to establish a foundation of comparison for results from future planned nutritional studies with the hide beetle, its life history was reexamined under controlled conditions; results are herewith presented.

MATERIALS AND METHODS

The stock colony of *Dermestes maculatus* consisted of descendants of specimens collected in Iowa at a soybean processing plant in the fall of 1946. The pests were apparently feeding and developing on fish meal stored in the same building. Techniques for rearing this insect in sufficient numbers for experimental purposes have been described by Scoggin and Tauber (1949).

Sardine fish meal was the basic dietary medium; contents of special diets are listed later.

Larvae hatching within a period of twelve hours prior to the beginning of a test were confined individually with one gram of medium in 25 x 50 millimeter shell vials. Vials were stoppered with cotton plugs. If water was added to the culture daily, cotton plugs were replaced by gauze-covered screen caps on the fifth or sixth day after the larvae had hatched. With increased moisture content of food, larvae were larger and more active. The prepupal instar, when the urge of migration was particularly evident, could tunnel through the cotton plug. Water was added to the medium by means of a pipette; the amount added was constant and was accurately determined. Each test, with one exception of 50 larvae, consisted of 25 individuals. Examinations were made

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daily to determine molts. Only those individuals emerging as normal adults were included in computation of larval and pupal developmental periods.

Tests insects were reared in a cabinet maintained at 32±1° C. and 70% relative humidity. Humidity was regulated by potassium hydroxide solutions as recommended by Buxton (1931).

INFLUENCE OF MOISTURE ON DEVELOPMENT

Sardine fish meal, as supplied by the dealer, had a moisture content of 6 to 7%. In equilibrium at 70% relative humidity, water content was increased to 7.5%. Three additional concentrations of moisture were tested with proper amounts of distilled water added. Results are summarized in Table I. All adult females coming through the tests produced fertile eggs.

TABLE I

LARVAL AND PUPAL DEVELOPMENT OF *Dermestes maculatus* WITH VARYING MOISTURE CONTENT OF SARDINE FISH MEAL

Percent moisture content of food:	7.5	10.5	15.9	46.0
Initial number of larvae	50	25	25	25
Number pupating	29	24	24	17
Number adults emerging	29	24	24	12
Larval time in days, range	44–91	19–38	18–37	15–25
mean	65.0	27.4	26.9	19.7
Larval time of ♀'s in days, range	45–90	23–38	20–37	20–25
mean	67.8	28.0	27.9	22.5
Larval time of ♂'s in days, range	44–91	19–36	18–31	15–21
mean	62.8	26.9	26.3	19.1
Number on instars	9–13	6–8	6–8	5–7
Pupal time in days	5–6	5–6	5–6	5–6
Ratio males : females	16 : 13	13 : 11	15 : 9	10 : 2
Mean wt. females (mg.)	24.6	40.6	37.1	32.4
Mean wt. males (mg.)	18.1	35.1	30.4	29.2

Development at 7.5% water content

Because of a high mortality when larvae were reared on fish meal with a moisture content as low as 7.5%, this test was repeated so that a total of 50 larvae was initially concerned. Of the 50, 29 pupated and emerged as normal adults. Larval development comprised 8 to 12 stadia of varying duration, prior to a prepupal stadium characteristic of all larvae. During the prepupal stadium, the larvae were at first restless, only to become lethargic and assume an "arched" position about four days before pupation. Pupation thus occurred after 9 to 13 instars.

The first stadiums lasted 2 to 6 days; the second, 5 to 10; the third, 4 to 9; the fourth through the eighth, 4 to 7; the ninth, 4 to 9; the tenth, 5 to 7; the eleventh and twelfth, 6 to 8; and the prepupal, 8 to 15 days. Of 29 individuals resulting from the started 50, 8 pupated after 9 instars; 9, after 10 instars; 5, after 11 instars; 4, after 12 instars; and 3, after 13 instars. Larval time was 44 to 91 days, with a mean of 65.0

days. Because females require a longer time for development, larval time for the sexes is calculated separately in Table I. Females are also consistently larger and heavier than males. Both sexes pupated after as few as 9 or as many as 13 instars; thus the number of instars is not related to sex. Pupal time was 5 to 6 days, with all pupae developing into normal adults.

Development at 10.5% water content

Of 25 larvae beginning at this moisture level, 24 normal adults resulted. The first, second, and third stadia lasted 2 to 3 days; the fourth, 2; the fifth, 2 to 3; the sixth, 3 to 4; and the prepupal stadium, 8 to 25 days. Of the 24 individuals, 5 pupated after 6 instars; 18, after 7 instars; and 1, after 8 instars. Larval time was 19 to 38 days with a mean of 27.4 days; pupal time was 5 to 6 days with all pupae developing.

Development at 15.9% water content

The first stadium was of 2 to 4 days duration; the second, 1 to 2; the third and fourth, 2; the fifth, 2 to 3; the sixth, 3 to 4; the seventh, 3; and the prepupal stadium, 8 to 23 days. Of 24 individuals reaching maturity, out of an initial 25, 5 pupated after 6 instars; 18, after 7 instars; and 1, after 8 instars. Larval time was 18 to 37 days with a mean of 26.9 days; pupal time was 5 to 6 days with all pupae developing.

Development at 46% water content

Even though this medium was changed daily, with a moisture content of 46%, a number of larvae were killed by molds, as were a few pupae. The first stadiums lasted 2 to 3 days; the second, 1 to 4; the third, 1 to 2; the fourth and fifth, 1 to 3; the sixth, 3; and the prepupal stadium, 7 to 16 days. Of the 12 adults resulting, 2 pupated after 5 instars; 9 after 6 instars; and 1, after 7 instars. Larval time was 15 to 25 days with a mean of 19.7 days. Of 17 pupae, 12 emerged after a pupal time of 5 to 6 days.

DEVELOPMENT OF D. MACULATUS ON VARIOUS MEDIA

The increasing popularity of this dermestid as a test insect in entomological research portends the need for suitable, but variable, diets on which this insect may be cultured. As a small beginning in this direction, larvae were confined with various widely different kinds of media. Data are summarized in Table II. All adult females resulting from the tests produced fertile eggs.

Development on soybean meal

Because this dermestid species was collected at a soybean processing plant, it was of interest to know whether larvae could develop on a diet of soybean. The first sample of soybean meal analyzed approximately 43% protein and 4.5% fat. All efforts to rear *D. maculatus* on the unaltered meal were futile. First stage larvae did not develop; and second or third stage larvae started on fish meal and transferred to this soybean meal were all dead within 30 days. A few larvae molted but not one reached the prepupal instar. Addition of 7% moisture to this low-lipid soybean meal did not alter it sufficiently to support larval development.

Larvae were also confined with a sample of oily soybean meal which analyzed 32% protein and 9% fat. Larvae did not develop on the unaltered meal, but addition of 7% moisture made it possible for 9 of 25 larvae to develop. Of the 9 individuals, 2 pupated after 5 instars; 3, after 6 instars; 3, after 7 instars; and 1, after 8 instars. Larval time was 25 to 36 days with a mean of 29.4 days, and pupal time was 5 to 7 days.

Development on modified complete food mixture

A modified "complete food" mixture was utilized as a regimen. The mixture consisted of ground yellow corn meal, 4 parts; whole wheat flour, 2 parts; whole milk powder, 2 parts; dried powdered yeast, 1 part; wheat middlings, 1 part; and bran, 1 part. This dry food mixture permitted only a small percentage of seeded first stage larvae to develop.

TABLE II

LARVAL AND PUPAL DEVELOPMENT OF *Dermestes maculatus* ON VARIOUS MEDIA WITH 7 PERCENT MOISTURE ADDED

Medium	Oily soybean meal	Modified complete food mixture	Casein, yeast, cholesterol medium	Fructose, yeast, cholesterol medium
Initial number of larvae	25	25	25	25
Number pupating	11	24	24	17
Number adults emerging	9	22	24	8
Larval time in days, range	25–36	19–38	21–30	21–26
mean	29.4	24.5	25.5	22.9
Larval time of ♀'s in days, range	25	19–38	21–30	21–26
mean	....	24.1	26.6	23.4
Larval time of ♂'s in days, range	25–36	20–36	22–29	21–23
mean	30	24.9	24.8	22
Number of instars	5–8	6–9	6–7	6
Pupal time in days	5–7	5–6	5–6	5–6
Ratio males : females	8 : 1	12 : 10	14 : 10	3 : 5
Mean wt. females (mg.)	19.8	25.9	32.3	33.6
Mean wt. males (mg.)	18.6	21.1	25.1	26.6

Addition of 7% moisture allowed 22 of 25 larvae to develop into adults. Of the 22 individuals, 3 pupated after 6 instars; 14, after 7 instars; 3, after 8 instars; and 2, after 9 instars. The larval time varied from 19 to 38 days with an average of 24.5 days; pupal time was 5 to 6 days.

Development on high casein content diet

A high protein diet consisting of 30 parts vitamin-free casein, 3 parts dried yeast, and 1 part cholesterol was not a successful medium, when dry. Addition of 7% water to this mixture permitted 24 of 25 test larvae to develop and emerge as adults. Of 24 larvae, 12 pupated after 6 instars; and 12 after 7 instars. Larval time ranged from 21 to 30 days with a mean of 25.5 days. Pupal time was 5 to 6 days.

Development on fructose, yeast diet

Addition of moisture to a regimen of 49 parts fructose, 50 parts dried yeast, and 1 part cholesterol supported rapid development of *D. maculatus*, but the physical characteristics of the mixture were not favorable to survival. This medium absorbed moisture to such an extent that the resulting gummy texture prevented many individuals from emerging. All 8 adults from the 25 test larvae pupated after 6 instars. Larval time was from 21 to 26 days with an average of 22.9 days. The pupal period lasted from 5 to 6 days. Male : female ratio was 3 : 5. Of the eight test conditions described in this investigation, this fructose-yeast-cholesterol diet is the only one which produced more females than males in the emergents.

DISCUSSION

Examination of results in Table I indicates that, in general, increased moisture content of sardine fish meal, even up to 46%, was beneficial to the hide beetle in reducing the number of instars, and in reducing duration of larval development. However, at 46%, eventual emergence was only about 50% of that at intermediate food moisture levels (between 10 to 15%), and body weight of females was approximately 20% less than that reached by females coming through on the 10.5% moisture meal. Although it is known that these smaller females produced fertile eggs, no attempt was made in these experiments to measure the egg-laying capacity of females of different size.

Possibly, an interesting biological trend is coming into view in Table I, in the shift in ratio of male to female emergents. As food moisture increased, especially up to the 46% water content, fewer females appeared among surviving adults. Although sample size of survivors is rather small in these experiments, this effect on differential survival of sexes may be significant and is worthy of additional tests. In connection with this point, a glance at Table II also shows that the sex ratio of this beetle may be upset by factors other than high water content of the food. Survivors on the oily soybean meal were predominately males.

Of various criteria examined, duration of pupal life seems least affected by alterations of water and nutrient contents of food. It seems that if a larva manages to reach the pupal stage, metamorphosis proceeds at a regular rate, regardless of the nutritional history of preceding stages. This, also, is a point of interest for further exploration inasmuch as it seems physiologically reasonable to assume that quality and quantity of food reserves, stored for maintenance of metamorphic activities, would be altered by the dietary fractions available during larval life.

COMPARISON WITH PREVIOUSLY PUBLISHED RECORDS

The time required for the larval and pupal development of *D. maculatus* has been reported by several investigators. Riley (1885) reared this insect with "plenty of food," at a temperature of 68° to 78° F., and reported that larvae molted 6 times and passed through 7 larval stages. He was convinced that, under conditions of warmth and sufficient food, larval time would be shortened to 2 or 3 weeks;

whereas under unfavorable winter conditions larval time would be not appreciably less than 45 days.

Illingworth (1918) reported that larvae of *D. maculatus* passed through 7 stages under the tropical conditions of Hawaii. He found that after the sixth molt the larvae were fully developed and immediately sought a place to pupate.

Kimura and Takakura (1919), in Japan, recorded 6 larval molts over approximately 50 days. Grady (1928) found some larvae molted 10 or 11 times. He reasoned that excess molts were due to a food deficiency. The average number of molts was 7 with a larval period of about 44 days.

Kreyenberg (1928) reared four individuals to adulthood. The three resulting males molted 6 times as larvae; while the one female had molted 7 times. He was inclined to believe that females consistently passed through one more instar than the males.

In New Zealand the period for oviposition to adulthood under "favorable conditions" was 6 to 8 weeks (Clark, 1929); in South Africa the larval period required from 35 to 238 days (Smit, 1934). Walker (1944) reported that larvae of the "hide beetle" remained in larval stages for about 6 weeks and molted 6 times at 5 to 8 day intervals.

Gay (1938), using *D. maculatus* as a test insect in nutritional studies, found that larval stages required an average of 41.4 days when reared on fish meal with 11 to 12 percent moisture content. He found that the number of instars depended on the nutrition provided.

Utilization of metabolic water by *D. maculatus* was investigated by Fraenkel and Blewett (1944). With decreasing moisture content of food, the larval period and number of molts increased. Depending on moisture content of the food, larvae pupated after the fifth to twelfth molt.

Riley (1885) reported the pupal stage of *D. maculatus* lasted 14 days, but conditions of temperature and humidity were not stated.

The pupation period has been given by other workers as follows: 5 to 12 days (Kimura and Takakura, 1919); 7 to 8 days (Grady, 1928); 15 days (Smit, 1934); and 10 to 11 days (Illingworth, 1918, and Walker, 1944).

SUMMARY AND CONCLUSIONS

1. Larval and pupal development of *Dermestes maculatus* was measured on four levels of moisture content of sardine fish meal; and on various other diets, with or without the addition of 7% moisture. All trials were conducted at 32° C., and 70% R.H.

2. With one exception of 50 larvae, 25 newly hatched larvae were maintained individually, for each experimental variable, in vials with 1 gram of food, changed as required, sometimes daily.

3. With the addition, to sardine meal, of sufficient water to bring the moisture content up to approximately 10 to 15%, larval mortality was lowered; the number of larval instars, and duration of larval development decreased; and larger adults emerged.

4. At the highest moisture level tested (46%), some mortality resulted from mold growths, but, discounting these deaths, such a moist medium was associated with the production of female beetles

averaging 20% smaller than those reared with 10.5% moisture in the food.

5. At the 46% moisture level, larval development was shorter than that on any other medium tested; and the number of instars tended to be less.

6. As water content of sardine meal was increased to higher levels, especially at 46% moisture, the sex ratio was disturbed, with fewer female adults emerging.

7. Preliminary experiments with various other diets demonstrated that plain soybean meal would not support development of the hide beetle.

8. Results with a few "synthetic" diets showed that mixtures with high protein content, some of which (casein) was of animal origin, would support development, but the emergents were as much as 25% lighter in weight. These smaller females layed fertile eggs.

9. A high yeast (50%), high fructose (49%) mixture, plus 1% cholesterol, supported rapid larval development, but became so gummy from absorbed moisture that high larval mortality resulted.

10. The number of larval instars of individuals on the same medium was inconstant; this was not related to sex.

11. Females required a slightly longer time for larval development; and female adults were heavier than males. These relationships were not dependent on moisture or nutrient contents of the diets tested in the present series.

12. Duration of pupal period was the most stable of the criteria used to measure effects on development of the hide beetle.

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THE PENETRATION, DISTRIBUTION AND METABOLISM OF ORGANIC PHOSPHATES IN THE AMERICAN ROACH, PERIPLANETA AMERICANA (LINN.)

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INTRODUCTION

Roan *et al.* (1950 reported the results of studies with P^{32} labelled organic phosphates. The effect of solvents on the permeabilities of these compounds, the rates of distribution through the tissues of the American roach and the rates of excretion of these compounds by the same insect were presented in the above mentioned paper. The investigation under review represents a continuation of these studies.

The compounds synthesized* with P^{32}† were tetraethyl pyrophosphate (TEPP), diethyl phosphoric acid, tetra-*n*-butyl pyrophosphate (T*n*BPP), and 0, 0-diethyl-0, *p*-nitrophenyl phosphate (para-oxon), while 0, 0-diethyl-0, *p*-nitrophenyl thiophosphate (parathion) was synthesized from radioactive phosphorus pentasulphide.† Parathion and diethyl phosphoric acid were not used in the previous work.

The details of the chemical syntheses of the compounds used are not presented here. Physical data such as index of refraction, density and boiling points of the radioactive compounds were not determined. However, such data obtained from preliminary syntheses using the stable isotope indicated a purity of greater than 90 percent for all the compounds used.

EQUIPMENT AND TECHNIQUES

The test insects in these studies were adult American roaches of both sexes, laboratory reared. Acetone was the solvent used for all the compounds. Topical applications were made in measured doses and applied in a manner described by Trevan (1922) on the cervical membrane. Every precaution was taken to evaporate the solvent from the administered drop before the insect was released. This measure precluded the possibility of the insect ingesting any of the topically administered dose of insecticide. To study the effects of ingestion of these compounds, dosages were administered by placing the blunt needle of a microsyringe between the maxillary lobes and thereby evoking a chewing and swallowing reflex. When about 3 mm. of the end of the needle was thus introduced into the pharynx of the insect the required dosage of the compound was released.

A freezing microtome was used for the preparation of the sections for radioautography. Kodak nuclear track plates, type NTB, 25 mm.

*The authors are indebted to Victor Chemical Works and especially Dr. A. D. T. Toy for their advice and recommendations in the synthesis of these compounds.

†The radioactive materials were obtained from Oak Ridge National Laboratory, Division of Carbide and Carbon Chemical Corp., on authorization from the Atomic Energy Commission.

x 75 mm., of 50 micron emulsion thickness and with a protective coat of gelatin, were used for the preparation of radioautographs. The developer (D_{19}) and a F_5 fixing bath were used as recommended by Eastman Kodak Company, New York, for the development and fixation of the NTB films. All darkroom work was conducted under a Wratten Series 2 lamp.

For all assays a thin-walled beta counter, Radiation Counter Laboratories, Mark 6 Model 3, was employed. The counter assembly was placed in a lead shield. The scaler used was a Model 163, obtained from Nuclear Instrument and Chemical Corporation. A Model T dual timer, obtained from the same source, was used with the scaler. This combination permitted the preselection of the counting time and the counting rate which could be adjusted according to the strength of the P^{32} at the time of the assay. Aluminum sample pans, Radiation Counter Laboratories Mark 5, Model 1, were used for the preparation of samples for counting and discarded after use.

The colorimetric method of Hestrin (1949) for the determination of acetycholine was employed to investigate the stability of TEPP after its entry into the body of the roach. A Coleman Junior spectrophotometer was used for colorimetric studies in this procedure.

RADIOAUTOGRAPHY

In their investigations on the distribution and utilization of radiophosphorus in four species of insects including the German roach, Lindsay and Craig (1945) either prepared (a) frozen sections after gelatin embedding and fixation in 10 percent formalin, or (b) paraffin sections after chloroform clearing and fixation in Susa's fluid. Neither of these two procedures could be adopted in the present work because of the solubility of TEPP in the various substances used. Russel *et al.* (1949) describe a technique for the study of the distribution of P^{32} in plant tissue. The plant tissue was frozen to $-170°$ C. in isopentane and then transferred to absolute alcohol saturated with lead acetate at $-70°$ C. The P^{32} was precipitated in the tissues as insoluble phosphate before sectioning. Holt *et al.* (1949) applied this technique to animal tissue without success. In the present investigation roaches, parlayzed by TEPP, were sectioned directly by the freezing technique described by Adamstone *et al.* (1948).‡ Sections were cut at 25 micron thickness. In preliminary work the sections were kept frozen throughout the period of exposure of the NTB film. This was done as follows: The frozen section was transferred on to a microscope slide cooled by dry ice. In a darkroom a cooled NTB film plate was placed over the section, and the microscope slide and film plate were held in position by binding with scotch tape. The entire preparation was placed in a thick-walled metal container, previously cooled in dry ice. The container was held in a refrigerator for five or six days. At the end of this period of time the NTB film was developed and fixed as recommended by Eastman Kodak Company, while the section was fixed in 95 percent alcohol as it

‡The authors are deeply indebted to Dr. F. B. Adamstone, Head of the Department of Zoology, University of Illinois, and Mr. C. A. Blomquist, of the same department, for much guidance and advice on techniques with the freezing microtome.

thawed. Although this procedure gave excellent radioautographs, more often than not parts of the section were lost by adhesion to the NTB film plate. An alternative procedure was therefore adopted. The frozen section was transferred on to a microscope slide which had a thin coating of Mayer's albumen. While the section thawed the slide

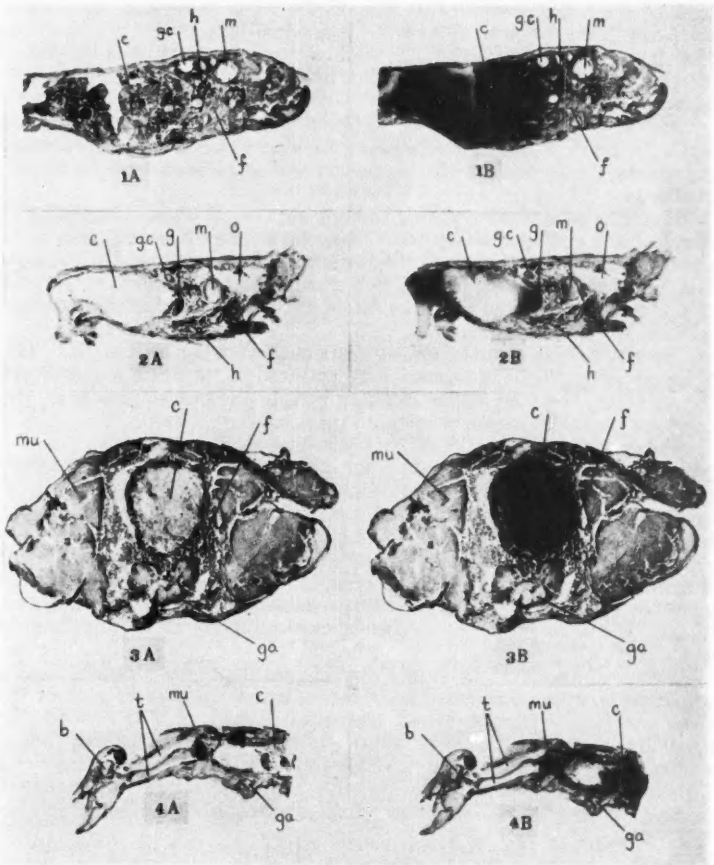

FIG. 1. Sections of roaches paralyzed by a lethal dose of TEPP on cervical membrane. 1A and 1B L. S. Abdomen. 2A and 2B L. S. Abdomen. 3A and 3B T. S. Thorax. 4A and 4B L. S. Head and Thorax. The A series represent the sections as such; the B series represents the sections with the appropriate radioautographs superimposed.

Explanation of abbreviations: b. cerebral ganglion, c. crop, f. fat body, g. muscles of gizzard, g.c. gastric caecum, ga. thoracic ganglion, h. hind gut, m. mid gut, mus. thoracic muscle, t. air sacs in neck.

was held under a Sylvania lamp for about 15 seconds. This served not only to fix the tissue but also to dehydrate it completely. A NTB film plate was placed against the dehydrated section in a darkroom. The preparation was wrapped in black opaque paper and left at room temperature for five to six days. The duration of exposure of the NTB film depended on the strength of the P^{32} in the section at the time of its preparation. The film plates were developed as already mentioned, while the sections were stained in haemotoxylin and eosin. The distribution of TEPP in the various tissues was studied by superposition of the radioautograph on the corresponding section and examination through a microscope.

Figure 1 shows a series of sections of roaches paralyzed by the action of TEPP. Series A represents the sections as such while Series B represents the sections with the corresponding radioautographs superimposed.

Examination of 1B, a longitudinal section of the abdomen of a female roach and comparison with 1A shows that the heaviest concentration of TEPP is in the crop. Slight traces of the radioactive material are to be found in the midgut and gastric caeca. Vague indications of the presence of TEPP in the fat body, muscles and hindgut where seen on the radioautograph.

2B shows the same trend of distribution of TEPP as 1B. The presence of radioactive material in the midgut is more apparent in this section however. The radioautograph showed no trace of the presence of TEPP in the ovary.

Comparison of 3A and 3B, a transverse section of the thorax of a paralyzed roach, shows again that the crop contains the heaviest concentration of TEPP. Very vague traces of the presence of TEPP are apparent in the muscles and fat body. No trace of radioactive material is apparent in the thoracic ganglion.

4A and 4B represent a longitudinal section of the head and thorax. The heaviest concentration of TEPP is in the crop. Faint traces of the presence of TEPP are apparent in the muscles while there is no trace of TEPP indicated either in the cerebral ganglion or in the thoracic ganglia.

From all the sections studied by radioautographic methods it was apparent that the crop selectively concentrates the greater part of the dose of TEPP from the blood, while the passage of TEPP down the alimentary canal is a very slow process. The Malpighian tubes, contrary to expectation, do not take up any appreciable quantity of TEPP from the blood. The quantity of TEPP entering the central nervous system is too small to cause a perceptible darkening of the NTB film.

TISSUE ASSAY

To obtain quantitative data of the tissue concentrations of the compounds being studied, roaches at various stages after treatment were anaesthetized with carbon dioxide gas and then killed by plunging them into acetone. The acetone also served to wash off any of the radioactive material applied that had not penetrated into the body of the insect. Anaesthesia was used before killing because if plunged

into acetone a normal roach regurgitates some of its crop contents, which in the case of treated roaches carry high concentrations of the radioactive material. Tissues of 5 treated roaches were dissected out for each time interval, rinsed in water, and dissolved in minimum amounts of nitric acid. One millilitre aliquots of the nitric acid solutions were placed in aluminum sample pans and evaporated to dryness under a 150 W. Sylvania spotlight. Blood samples taken from treated roaches were carried through the same procedure before assay. The samples in the pans were then assayed with a beta counter. The count for the total dose administered per roach was obtained by treating five roaches and dissolving them immediately in nitric acid without washing in acetone. An aliquot of this solution was then assayed. Background counts were taken during each working day and the

TABLE I

DISTRIBUTION OF PARA-OXON IN TISSUES OF ROACHES AFTER TOPICAL APPLICATION OF 5 μg[1] PER ROACH ON THE CERVICAL MEMBRANE

Tissue	10 min.		15 min.		30 min.	
	cpm/R[2]	μ g	cpm/R	μ g	cpm/R	μ g
Foregut	787.2	1.2	1536	2.3	1904	2.91
Midgut	37.31	0.056	48	0.07	56	0.085
Hindgut	28	0.041	41	0.061	40	0.06
Fat	51	0.075	54	0.08	67	0.11
Central nervous system	10	0.015	15.3	0.021	17	0.025
Thoracic muscle and legs	187	0.285	244	0.37	261	0.39
Blood[3]	373	0.55	396	0.61	285	0.57
Remainder	236.8	0.35	273.2	0.415	299	0.45

[1]Total count for 5 μg dose, 3284 counts per minute.
[2]Count per minute per corresponding tissue of one roach.
[3]Count for the blood of a single roach was computed from an assay of a sample of 30–60 mg. of blood, on the basis that in a roach the blood represents 20 percent of the body weight (Yeager and Munson 1949).

necessary corrections made in the counts obtained from tissue assays. The results presented here on the distribution of the compounds used are not in complete agreement with those presented in the previous work because of the use of lethal instead of sublethal doses of the compounds in most cases.

Table I gives the data of the distribution of para-oxon in a roach after treatment with 5μg of this substance on the cervical membrane. The data show that the penetration of para-oxon into the body of the roach is an extremely rapid process. Within ten minutes as much as 51 percent of the administered dose has entered the roach, while at paralysis about thirty minutes after treatment about 92 percent has penetrated the body wall of the insect. The blood is the chief medium of transport of para-oxon within the body of the roach and this is true

of all the organic phosphates studied. Concentration of the toxicant by the crop is extremely rapid and selective. The amount of para-oxon entering the central nervous system is very small indeed, but as might be seen later it is probably many times that required for the inhibition of the cholinesterase of this system.

Table II represents the data of the distribution of parathion in the tissues of a roach after treatment with 5μg. of this substance on the cervical membrane. Compared with that in Table I these data show that parathion is relatively much slower than para-oxon in its penetration and distribution in the roach. The concentration of parathion by the crop although relative to that of the other tissues is quite marked, when compared with the same phenomenon for para-oxon appears small. The accumulation of parathion in the central nervous system of a treated roach is a very slow process. Even at the end of four hours, by which time the treated insect is prostrate, the amount of parathion in the central nervous system is only about three-fourths the amount found in this system at the end of thirty minutes in a roach treated with para-oxon. This probably, at least in part, explains the slower action of parathion.

The distribution of 5μg doses of diethyl phosphoric acid and TEPP, administered on the cervical membranes of roaches, is presented in Table III. The data show that diethyl phosphoric acid follows the same general pattern of distribution in the tissues of the roach as does TEPP and the other organic phosphates studied. This has also been found to be true for T*n*BPP, which, like diethyl phosphoric acid, is non-toxic to the roach. At the end of ninety minutes approximately as much diethyl phosphoric acid has entered the central nervous system of a treated roach as has TEPP sixty minutes after treatment of a roach, while this quantity is in excess of the amount of para-oxon found in the same system of a roach knocked down by the latter compound. These facts are particularly interesting because of Hanson's (1947) suggestion that TEPP hydrolyzes in the body of the insect to give diethyl phosphoric acid which then interferes with the synthesis of adenosine triphosphate, and Burgan's (1949) view that cholinesterase inhibition by the phosphate esters is effected by a dialkyl phosphorylation of the enzyme. *In vitro* studies showed that diethyl phosphoric acid does not inhibit cholinesterase while the data on Table III show clearly that diethyl phosphoric acid enters both the central nervous system and the muscles in quantities comparable to those for TEPP and larger than those for para-oxon.

Doses of TEPP as large as 40 μg can be administered to a roach orally without the appearance of any toxic symptoms in over 95 percent of the treated insects. Orally administered doses of para-oxon or parathion on the other hand act very differently. A dose of 5 μg of either of these compounds when ingested by a roach evokes toxic symptoms as rapidly as a topically administered dose of the same size. It was therefore interesting to investigate the cause of these differences. Table IV gives the data of the distribution of the three compounds in roaches after oral administration of 5 μg of each compound. For all three compounds there is a progressive decrease in the concentration of the toxicant in the crop while there is a gradual increase in the con-

TABLE II

DISTRIBUTION OF PARATHION IN TISSUES OF ROACHES AFTER TOPICAL APPLICATION OF 5 μg[1] PER ROACH ON CERVICAL MEMBRANE

Tissue	15 min.		30 min.		60 min.		150 min.		240 min.	
	cpm/R	μg	cpm/R	μg	cpm/R	μg	cpm/R	μg	cpm/R	μg
Foregut	141	0.625	163	0.72	170	0.76	206	0.915	297	1.30
Midgut	14	0.0625	14.6	0.064	32.25	0.140	53.1	0.236	63	0.280
Hindgut	4	0.0175	4.3	0.019	13.4	0.060	48	0.213	42.8	0.19
Fat	7	0.031	8.4	0.37	16.75	0.075	17.3	0.077	29	0.13
Central nervous system	2	0.008	2.6	0.0115	2.7	0.012	2.9	0.0128	4	0.017
Thoracic muscles and legs	44.12	0.19	57.5	0.255	64.5	0.29	67.5	0.30	72.25	0.32
Blood	81	0.36	83.25	0.37	95.5	0.424	127	0.565	108	0.45
Remainder	27.2	0.12	46.2	0.205	49.5	0.22	53	0.235	61.12	0.27

[1]Total count for 5 μg dose, 1125 counts per minute.

TABLE III

DISTRIBUTION OF TEPP[1] AND DIETHYL PHOSPHORIC[2] ACID IN TISSUES OF ROACHES AFTER TOPICAL APPLICATION OF 5 μg OF THE SUBSTANCES ON THE CERVICAL MEMBRANE OF ROACHES

Tissue	TEPP		Diethyl Phosphoric Acid					
	60 min.		30 min.		60 min.		90 min.	
	cpm/R	μg	cpm/R	μg	cpm/R	μg	cpm/R	μg
Foregut	1056	2.40	244	1.02	344	1.40	409	1.70
Midgut	74	0.167	37	0.15	51	0.210	72	0.29
Hindgut	65	0.145	21	0.85	29	0.120	45	0.185
Fat	78	0.171	9	0.037	16	0.066	30	0.122
Central nervous system	18	0.041	4	0.016	7	0.029	9	0.37
Thoracic muscles and legs	180	0.410	45	0.185	60	0.25	79	0.328
Blood	196	0.450	78	0.32	84	0.35	80	0.33
Remainder	161	0.365	40	0.165	52	0.215	61	0.25

[1]Total count for 5 μg dose of TEPP, 2200 counts per minute.
[2]Total count for 5 μg dose of diethyl phosphoric acid, 1204 counts per minute.

TABLE IV

DISTRIBUTION OF INGESTED DOSES OF TEPP, PARA-OXON AND PARATHION AFTER ADMINISTRATION OF 5 μg OF THE SUBSTANCE PER ROACH ORALLY

TISSUE	TEPP[1]						PARA-OXON[2]				PARATHION[3]					
	1 hr.		8 hrs.		24 hrs.		15 min.		30 min.		1 hr.		2 hrs.		3 hrs.	
	cpm/	μg	cpm/	μg	cpm/	μg	cpm/	μg	cpm/	μg	cpm/	μg	cpm/	μg	cpm/	μg
Foregut	3008	4.35	2876	4.05	2560	3.65	1406	3.09	960	2.10	2836	3.95	2106	2.91	1605	2.23
Midgut	320	0.463	272	0.380	448	0.65	123	0.27	134	0.30	168	0.234	213	0.295	240	0.334
Hindgut	101	0.145	212	0.305	79	0.091	98	0.215	145	0.315	137	0.190	240	0.334	281	0.391
Fat	21	0.03	32	0.046	86	0.11	46	0.10	60	0.132	143	0.2	186	0.255	200	0.275
Central nervous system	4	0.005	6	0.007	14	0.02	5	0.011	14	0.03	10	0.0135	19	0.026	28	0.039
Thoracic muscle and legs	32	0.04	60	0.075	206	0.30	189	0.415	290	0.61	176	0.24	317	0.44	480	0.665
Blood	42	0.06	56	0.08	83	0.105	103	0.225	187	0.4	78	0.105	91	0.126	112	0.155
Remainder	24	0.03	96	0.12	312	0.45	229	0.5	481	1.05	316	0.44	425	0.590	645	0.853

[1]Total count for 5 μg dosage 3452 per minute.
[2]Total count for 5 μg dosage 2274 per minute.
[3]Total count for 5 μg dosage 3591 per minute.

centration of these materials in the midgut and hindgut with time. This fact would suggest that the crop epithelium is the main site through which the ingested doses diffuse into the blood and thence the rest of the body. The data show that the rate at which TEPP leaves the crop to enter the blood stream is markedly slower than that for para-oxon and parathion. Furthermore data in Table VII show that the radioactive material in the blood stream of a roach treated orally with TEPP does not inhibit cholinesterase. Thus the extremely slow rate of outward diffusion of an orally administered dose of TEPP and the fact that what enters the blood stream under such conditions is a metabolite of TEPP, most probably diethyl phosphoric acid, accounts for the absence of toxic symptoms in a roach treated orally with TEPP. Another fact that is apparent from these data is the differential permeability of the crop to the three compounds, from the crop into the blood stream. In the case of TEPP this is a very slow process, while for para-oxon and parathion it is definitely very rapid so that lethal accumulations result in the vulnerable tissues before any detoxifying mechanism can produce its effects on an orally administered dose.

Burgan (1949) suggested that the inhibition of cholinesterase by the organic phosphates was brought about by a dialkyl phosphorylation of the enzyme itself. If this is so the application of tracer techniques with purified enzyme preparations should give valuable information on this point. Although no purified cholinesterase was available at the time, tests were carried out with homogenized roach nerve tissue in order to shed some light on this problem.

Each of ten roaches were treated topically with 5 μg of para-oxon. At knockdown the brains and nerve cords were dissected out in Belar solution (0.9% NaCl, 0.02% $CaCl_2$, 0.02% KCL, 0.02% $NaHCO_3$) and homogenized in 5 cc. of saline (0.15 M NaCl, 0.04 M $MgCl_2$, 0.025 M $NAHCO_3$) in a Potter-Elevhjem homogenizer. The homogenate was treated with an excess of 25 percent trichloroacetic acid and the precipitate was separated by centrifugation. After three washings with trichloroacetic acid solution and centrifugation, the precipitate was dissolved in 1 cc. of nitric acid and assayed for radioactivity. In a second test ten roaches were given the same treatment, the brains and nerve cords were removed at knockdown, wet ashed directly and assayed for radioactivity. In a third experiment the brains and nerve cords of ten untreated roaches were dissected out in Belar's solution and homogenized in 5 cc. of saline. To this homogenate 10 μg of para-oxon in acetone were added and the preparation was held for one hour with continuous stirring. The homogenate was then treated with an excess of trichloroacetic acid and the precipitate was carried through the same procedure as that for the precipitate in the first experiment. In the last test ten roaches were treated with 5 μg each of TEPP containing the stable isotope of phosphorus. At knockdown the brains and nerve cords were dissected out in Belar's solution and homogenized in 5 cc. of Saline. Then 10 μg of para-oxon in acetone were added to the homogenate, and the rest of the experiment followed the same procedure as that described for the third experiment. Tests on checks by the Hestrin method (1949) indicated complete inhibition of

Cholinesterase in all the above experiments. The data obtained from these tests is presented in Table V.

Although purified cholinesterase was not used in this test and the techniques adopted would not give highly accurate results, the data on Table V suggest that some chemical complexing occurs between the inhibitor and the substance of the nervous system, most probably with the cholinesterase. Although as much as 0.025 μg to 0.03 μg of para-oxon has entered the central nervous system of a roach by the knock-down stage, apparently only an average of 0.0006 μg of the substance is utilized in the inhibition of cholinesterase. As early as ten minutes

TABLE V

ASSAY OF NERVOUS TISSUE OF ROACHES AFTER *in Vitro* AND *in Vivo* TREATMENT WITH PARA-OXON

Tissue	Treatment	Counts per minute above background	μg para-oxon present per central nervous system
Trichloracetis acid precipitate of 10 brains and nerve cords	5 μg para-oxon per roach—topical	11	0.0008
10 brains and nerve cords	5 μg para-oxon per roach—topical	366	0.03
Trichloracetic acid precipitate of 10 brains and nerve cords	*in vitro* with 10 μg of para-oxon in acetone	13	0.001
Trichloroacetic acid precipitate of 10 brains and nerve cords	5 μg TEPP per roach—topical, *in vitro* treatment of brains and nerve cords with 10 μg para-oxon	4	0.0003

after the administration of a dose of 5 μ g of para-oxon in acetone on the cervical membrane of a roach about twenty-five times the dose required for cholinesterase inhibition is present in the central nervous system and by knockdown about forty times the required amount of inhibitor has entered this system (Table I). This delay of inhibition of the cholinesterase *in vivo* can be accounted for by the blocking action of the normal substrate, possibly acetyl-B-methylcholine (Metcalf and March, 1950), as shown by Burgan (1949) for TEPP.

STABILITY OF TEPP AFTER PENETRATION

In addition to determining the tissue distribution of some of the organic phosphates by radioactive tracer methods, tests were carried out to determine the ability of the blood or the intestinal tract to detoxify TEPP. The colorimetric method of Hestrin (1949) for the determination of acetylcholine was employed as an indication of the

inhibitory action of the blood or alimentary tracts of treated roaches. Roaches were treated by topical applications of TEPP in acetone or by ingestion of similar solutions. At various time intervals the treated roaches were sacrificed and blood samples withdrawn. Alimentary tracts were removed and homogenized in a 1 milliliter aliquot of rat brain homogenate. The rat brain homogenate was prepared by homogenizing a rat brain in 10 ml. of saline (0.15 M NaCl, 0.04 M $MgCl_2$, 0.025 M $NaHCO_3$). The resulting homogenate was diluted to 50 ml. with the same saline and stored in the refrigerator until needed. Fresh brain homogenates were prepared daily. The combined homogenates, *i.e.*, rat brain and roach tissue, were placed in shell vials and kept at room temperature for thirty minutes, to allow for reaction of the inhibitor in the roach tissue and the rat brain cholinesterase (Burgen, 1949) before addition of the acetylcholine substrate.

TABLE VI

INHIBITION OF RAT BRAIN CHOLINESTERASE BY BLOOD AND THE FOREGUT OF ROACHES AT KNOCKDOWN STAGE FOLLOWING TOPICAL APPLICATION OF 5μg DOSE OF TEPP IN ACETONE SOLUTION

Tissue or inhibitor used	μg TEPP present in tissue[1]	mg acetylocholine hydrolyzed in 30 minutes
Foregut	2.40	0
100 mg blood	0.25	0
10 TEPP	.....	0
Rat brain homogenate control	.....	1.81
Foregut from untreated roach	.....	1.81

[1]Determined quantitatively by radioactive tracers.

Thirty minutes were allowed after the addition of the substrate before the colors were developed and inhibition evaluated with a spectrophotometer. Preliminary experiments indicated that the amount of brain tissue used, *i.e.*, a 1 milliliter aliquot of a 50 milliliter rat brain homogenate, contained sufficient cholinesterase to hydrolyze in excess of 2–7 mg of acetylcholine in 30 minutes. In the tests reported here 2 ml. of 0.004 M acetylcholine in 0.001 N sodium acetate solution were employed as a substrate.

For each test a standard of acetylcholine and two checks, one of brain homogenate and one of brain homogenate plus the actual dosage of TEPP given one test roach were run. The acetylcholine standard was used to establish the optical density of the solution for each test since some decomposition was possible. The checks indicated the ability of the brain tissue to hydrolyze acetylcholine and the inhibitory actions of the applied dosage under *in vitro* conditions.

The data in Table VI give the results from topical applications of tetraethyl pyrophosphate in acetone solution to the ventral cervical membrane. The roaches were sacrificed at knockdown. The blood samples used in these tests were collected from roaches at this stage,

placed in water at 60° C. for 10 minutes. The foregut from a single roach was used for each test. The data in Table VI indicate that the inhibitory activity of the TEPP has not been lost either in the blood or in the foregut. It should be pointed out, however, that the amount of tetraethyl pyrophosphate required for *in vitro* inhibition of cholinesterase is extremely small. The data for inhibitory activity reported here are qualitative and indicate that changes, if any, that may occur in the TEPP after its entry into the body of the roach and subsequent concentration by the foregut are not extensive enough to destroy completely its anti-cholinesterase activity.

Fecal matter collected from roaches given sublethal topical applications of TEPP has been shown to contain a major part of the radioactivity of the original dose (Roan *et al.*, 1950). The radioactive material was found to be water soluble but insoluble in common organic

TABLE VII

INHIBITION OF RAT BRAIN CHOLINESTERASE BY THE INTESTINAL TRACTS AND BLOOD OF ROACHES 24 HOURS AFTER INGESTING 5 μ g OF TEPP IN ACETONE SOLUTIONS

Tissue or inhibitor used	μ g TEPP present in tissue[1]	mg acetylcholine hydrolyzed in 30 minutes
Foregut	3.65	0
Rest of gut	0.741	0
100 mg blood	0.058	1.80
5 μ g TEPP	.....	0
Brain homogenate control	.....	1.81
Alimentary tract of untreated roach	.....	1.81

[1]Determined quantitatively by radioactive tracers.

solvents. Water extracts of fecal matter from roaches given this treatment failed to inhibit rat brain cholinesterase. This would indicate that the decomposition of the TEPP occurs after it leaves the foregut and before it is voided with the feces.

As shown earlier, roaches can ingest large doses of TEPP, many times the lethal concentration, without showing symptoms of poisoning for over a month. The same could not, however, be said for parathion and para-oxon. It was therefore interesting to determine the fate of an ingested dose of TEPP with regard to its inhibitory activity. The data in Table VII give the results of such a study. These data indicate that the material present in the intestinal tract by ingestion is not detoxified. Although the blood contains small quantities of radioactive material it shows no anticholinesterase activity. This fact, together with the very slow rate of distribution of the radioactive material from the foregut as already stated accounts for the absence of any toxic symptoms in roaches given many times the lethal dose of TEPP by ingestion.

DISCUSSION

A simple technique has been described for the preparation of radioautographs of the tissue distribution of a substance soluble both in water and organic solvents normally used in the preparation of tissues for microtomy. Provided the tissue is not allowed to thaw several times during sectioning no histological damage, such as the rupture of cell boundaries and nuclear disintegration, takes place. Dehydration and fixation by heat did not cause a redistribution of the tagged compound in the tissues since the results obtained by this method are in close agreement with those obtained by assay with the beta counter.

In most insects the crop is regarded as a reservoir for food. In the Orthoptera, secretions from the midgut are said to be passed forward into the crop where much of the digestion occurs. In the roach, according to Sanford (1918) and Abbott (1926), some fat may be absorbed in the crop. Edimann (1922) showed that the crop, when set up as an osmometer, is completely impermeable to water. Thus, apart from its functions as a food reservoir and a locus of some digestive activity and fat absorption, no other role has been reported for the crop in the physiology of the insect. The results of these investigations clearly indicate that the crop plays a major part in the excretion of all the organic phosphates studied. A comparison of the rate of entry into the crop of these compounds from the blood stream in topical treatment, with the rate at which they leave the crop to enter the blood stream from an ingested dose shows that the former is spectacularly the more rapid process, especially when the concentrations of the materials on either side of the crop epithelium in the two cases is taken into account. Furthermore the penetration of the organic phosphates through the cuticle and body wall cannot be compared to their entry into the crop because the layers of tissue encountered in the latter process are in the reverse orientation of that encountered in the former. The phenomenon may most appropriately be considered analogous to the active absorption of excretory materials from the blood stream by the Malpighian tubes.

Studies of the penetration and distribution of the organic phosphates show that in the first ten to fifteen minutes their rate of entry into the body of the roach is very rapid in all cases. Both the active toxicants such as TEPP, para-oxon and parathion and the nontoxic members like diethyl phosphoric acid and T*n*BPP follow the same general pattern of distribution in the tissues of the roach so that the failure of diethyl phosphoric acid and T*n*BPP to evoke toxic symptoms in the roach is not due to anomalies in their penetration and tissue distribution but to certain intrinsic structural features of their molecules which do not characterize them as inhibitors of cholinesterase (Metcalf and March, 1949). The fact that diethyl phosphoric acid accumulates in the central nervous system in quantities comparable to those for TEPP and the other toxic phosphates studied, and that *in vitro* studies show that it does not inhibit cholinesterase is especially interesting because of Burgen's (1949) suggestion that inhibition of cholinesterase by the organic phosphates is caused by dialkyl phosphorylation of the enzyme. Thus for TEPP, diethyl phosphoric acid would have to be the direct intermediate concerned in this reaction. This point has already

been mentioned by Brauer (1949). Further evidence that dialkyl phosphorylation could not possibly be the process involved in cholinesterase inhibition is presented by the compound ethyl p-nitrophenyl benzene thiophosphate which inhibits cholinesterase (Metcalf and March, 1949) but could not possibly effect a dialkyl phosphorylation of cholinesterase.

Assays of well washed precipitates of homogenates of nervous tissue of the roach treated with para-oxon support the findings of Michael and Krop (1948) and Brauer *et al.* (1949) that the inhibition of cholinesterase by the organic phosphates is effected by a chemical combination of the inhibitor with the enzyme. The amount of phosphate ester involved in this reaction is extremely small in comparison to that which accumulates in the central nervous system by the time knockdown occurs. Almost immediately after topical treatment of a roach apparently many times the dose of toxicant required for inhibition of cholinesterase is present in the central nervous system. The delay in the appearance of symptoms of complete cholinesterase inhibition may be due to the blocking action of the normal enzyme substrate present in the nervous tissue and this is possibly most marked in the case of parathion.

SUMMARY

A simple method is described for the preparation of radioautographs for gross distribution studies of a tagged compound which is soluble both in water and all organic solvents used in techniques for the preparation of tissues for microtomy.

The penetration and distribution of TEPP, diethyl phosphoric acid, T*n*BPP, para-oxon and parthion in the tissues of the roach both after topical treatment and ingestion are discussed. All the compounds follow the same general pattern of distribution in the tissues of the roach with the crop acquiring the highest concentrations.

Diethyl phosphoric acid accumulates in the central nervous system in comparable or even larger concentrations than TEPP and para-oxon in topical treatments. Diethyl phosphoric acid does not inhibit cholinesterase *in vitro*. These facts together with others already mentioned are incompatible with Burgen's (1949) view that cholinesterase inhibition is the result of a dialkyl phosphorylation of the enzyme.

Data on the distribution of ingested doses of TEPP, para-oxon and parathion show that TEPP leaves the crop to enter the blood stream at a slower rate than do para-oxon and parathion. This in part accounts for the absence of any toxic effects in the roach from an ingested dose of TEPP.

Studies on the metabolism of TEPP after it enters the insect body both from ingestion and from topical application show that the greater part if not all the TEPP in the crop and the blood stream after topical treatment of a roach retains its anticholinesterase activity while after ingestion the TEPP in the crop is still active after twenty-four hours whereas the same cannot be said of the radioactive material in the blood after such a treatment.

Evidence is presented to support the work of Michael and Krop

(1948) and Brauer *et al.* (1949) which shows that in the inhibition of cholinesterase by the phosphate esters a chemical combination of enzyme with inhibitor takes place.

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PROCEEDINGS OF THE ALASKAN SCIENCE CONFERENCE OF THE NATIONAL ACADEMY OF SCIENCES, NATIONAL RESEARCH COUNCIL, WASHINGTON, NOVEMBER 9–11, 1950. 222 pages, 1 map. National Research Council, Bulletin 122. National Research Council, National Academy of Sciences, Washington, D. C. 1951. Price, $2.00.

The proceedings are given mostly in abstract form. There is material of interest to the medical entomologist scattered through these abstracts, and the amount of material of general biological interest is much more extensive.

—M. T. J.

"DUMÉRIL, 1798": A PROBLEM IN ENTOMOLOGICAL BIBLIOGRAPHY, WITH SPECIAL REFERENCE TO TETANOCERA (DIPTERA)

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Changes in established names for purely nomenclatural reasons are often annoying, have frequently caused strong opposition to rules of nomenclature, and have spurred on demands for suspension of such rules to preserve well-known usage. On the other hand, it is only too often true that the basic fault lies not with rules of nomenclature, but with taxonomists themselves. If the literature had been carefully studied and properly and accurately catalogued, they could often have avoided errors which years of repetition made habits with staunch defenders. In some cases, one must admit in all justice, the present situation is due to the operation, in past time of some principle that is not acceptable under modern rules of nomenclature, or to uncertainty or differences of opinion as to actual date of publication of a significant work. Yet with all due allowances, it is only too clear that cataloguers and revisers have a grave responsibility to go back to the original references and faithfully record the information found there.

The investigation of the obscure paper referred to as "Duméril (1798)" provides an excellent illustration of the above remarks, and also resulted in information that seems worthy of record.

For many years the literature of the order Diptera has contained citations for certain genera of flies credited to Constant Duméril (*Tetanocera*, *Echinomya*, and five less-well-known genera). The claim that these names were published in 1798 has provoked several controversies, and has affected the standing of some common genera. It is clear from the published discussions, however, that few if any dipterists have ever examined all the originals of the pertinent publications. It is also obvious, when one sees the originals, that the names have not always been cited in revisions and catalogues exactly as they were published, and that these errors or intentional emendations have been quoted over and over again without verification.

Since the names have been cited as of various dates from 1798 to 1809, and variously credited to Duméril and to Latreille, the pertinent literature was studied to determine the correct author and date, as well as to seek an explanation for the confusion that has existed. The generic name *Tetanocera*, which has been long and widely used for a common and characteristic genus of marsh flies of the family Sciomyzidae (called by some writers the Tetanoceridae), was the particular object of the search, and will serve as the major example.

Before proceeding with the chronology of Duméril's works, the confusion regarding *Tetanocera* can be shown by references to important works that have cited the name in different ways:

(1) *Tetanocera* credited to Duméril, 1798, Millin's Magasin.
Osten Sacken, 1900, Verhandl. Zool.-Bot. Gesell. Wien, 50: 450–451.
Aldrich, 1905, Catalogue N. Amer. Diptera, p. 579.
Hendel, 1908, Verhandl. Zool.-Bot. Gesell. Wien, 58: 64.
(2) *Tetanocera* credited to Duméril, 1801, Millin's Magasin.
Bezzi, 1908, Wien. Ent. Ztg. 27: 75–76.
Cresson, 1920, Trans. Amer. Ent. Soc. 46: 54 (as Tetanocere).
(3) *Tetanocera* credited to Latreille, 1804, Nouveau Dictionnaire.
Cresson, 1920, Trans. Amer. Ent. Soc. 46:54 (Revision of Nearctic Sciomyzidae).
Schulze, Kükenthal & Heider, 1936, Nomenclator animalium, gen. et subgen., p. 3421.
Neave, 1940, Nomenclator Zoologicus, vol. 4, p. 433.
Stone, 1941, Ann. Ent. Soc. Amer. 34: 414.
(4) *Tetanocera* credited to Latreille, 1805, Hist. Nat. Crust. et Ins.
Coquillett, 1910, Proc. U. S. Natl. Mus. 37: 613 (Type Species of North American Genera of Diptera).
(5) *Tetanocera* credited to Duméril, 1806, Zoologie analytique.
Hendel, 1900, Verhandl. Zool.-Bot. Gesell. Wien, 50: 319 (Revision of European species of *Tetanocera*).
Becker, 1905, Katalog Paläark. Dipteren, vol. 4, p. 62.
Sack, 1939, Sciomyzidae, in Linder's Die Fliegen der Palearktischen Region, Band V, Fam. 37, p. 48.
(6) *Tetanocera* credited to Latreille, 1809, Genera Crust. et Ins., IV.
Osten Sacken, 1878, Catalogue Diptera N. Amer., p. 177 (with the older name *Tetanocerus* Duméril 1801 [sic] in synonymy!).
Scudder, 1882, Suppl. List of Genera in Zool., p. 329.
(7) No mention of *Tetanocera* at all; *Tetanocerus* credited to Duméril, 1806.
Agassiz, 1845, Nomenclator Zoologicus, Diptera, p. 39.

CHRONOLOGY

(1) 1800 (An VIII of the French Revolution).—Cuvier, "Leçons d'Anatomie comparée." In volume 1, Duméril inserted what he later (1823, p. 264) called "les premières tentatives que j'ai faites de la classification, par familles naturelles, des genres d'insectes." Generic names are given both in vernacular (French) and Latin, arranged in tabular keys. No new genera of Diptera. Apparently published early in 1800, for its appearance was noted in the Journal de physique, vol. 50 (1800), issue of the month of germinal (March 21–April 19).

(2) 1800, October 24 (3 brumaire an IX).—Duméril presented his manuscript work on classification to the Société philomatique. In 1823 (p. vi), Duméril wrote as follows: "Ce travail, manuscrit de 266 pages, avoit titre: 'Les Insectes rangés par familles naturelles.' Il a été parafé par le secrétaire de la Société philomatique, enregistré sous le n. ° 414, séance du 3 Brumaire an IX (Septembre 1800)." This manuscript was never published separately; several extracts appeared (cf. items 3–6), and the entire work was finally incorporated into Duméril's "Zoologie analytique," 1806 (cf. item 11).

(3) 1800, November 21–December 20 ("frimaire an 9").—Duméril, "Exposition d'une Méthode Naturelle pour la classification et l'étude des insectes, présentée à la Société philomatique le brumaire an 9." Journal de physique, de chimie, d'histoire naturelle et des arts (Paris), 51: 427–439, 5 tables. Quarto.

As in the following paper, this is not a complete presentation of Duméril's classification, but only selected examples to illustrate his method, namely, a tabular key to the orders of insects, keys to the

families of "Les Coleoptères" and "Les Diptères," and keys to the genera of one family under each of those two orders. All order, family, and generic names are in the vernacular (French) only.[1]

The name "Tétanocère" is given in the tabular key to genera on page 438, and on the following page appears the entry: "Tétanocère. Ce sont les mouches désignées dans Fabricius, sous le nom de *nigripennis, marginata, reticulata, vesicularia, elata, clavata, planifrons, furcata, palustrata, cucularia*, etc."

The appearance of this "Exposition" in the Journal de physique seems to have been overlooked by entomological bibliographers. It is not listed in such standard works as Hagen's Bibliotheca Entomologica (1862) and the Index Litteraturae Entomologicae of Hagen, Horn, and Schenkling (1928), nor is it even mentioned in the list of Duméril's papers compiled by the Secretary of the Société entomologique de France (cf. item 17). We are fortunate that it is virtually identical with the paper in Millin's Magasin, and that it does not contain some validly proposed genera! The paper was discovered through a reference to it by Duméril (1823, p. 264) in reviewing his entomological work.

It may be added that this paper by Duméril may have special importance in a few genera, for it appears to be the first publication in which species are associated with some of the genera of Latreille (1796).

(4) 1800, December 21 (nivôse an 9).—Duméril, "Exposition d'une Méthode naturelle pour l'étude et la classification des Insectes, présentée à la Société philomatique le 3 brumaire an 9." Millin's Magasin encyclopédique (series 6), vol. 4, pp. 433–452, 5 tables. Octavo.

This work by Duméril has usually been cited as 1801, probably because of "an 9" fell in that year; however, the date "nivôse an 9" (nivôse = Dec. 21–Jan. 19) and the statement that the issues of this journal appeared on the first of each month, indicate Dec. 21, 1800, as the date of publication.

It may be noted that the title pages of issues at this time bore the statement "VI. Année, tome quatrième," with the imprint date at the bottom of the page "An 9." Considerable confusion has been caused in the past because "VI. Année" has been interpreted as the sixth year of the French Revolution, and hence 1798, instead of the sixth *series*. Cresson (1920, Trans. Amer. Ent. Soc. 46: 54–55) correctly explained this situation. Unfortunately, he caused further confusion by stating that Osten Sacken (1900, see later discussion) "refers to a special author's edition of the article dated 1798." Careful reading of Osten Sacken does not substantiate this statement; Osten Sacken stated only that the author's edition was said to be a "Separatausgabe" from an article in Millin's Magasin, vol. 4, p. 433 (1798). The date 1798 is that cited for the original article.

In the introduction, Duméril stated that this is a revision of his first "tentatives" inserted in Cuvier's work on comparative anatomy.

[1]In very few cases, the generic names appear to be in accepted Latin or Latinized form, but these cases involve names for which the Latinized and vernacular names are identical in form (e.g., *Lispe*). In view of the consistent use of the vernacular, these names must logically be regarded as being also in the vernacular at this time.

On pages 446–447, he gives the genera that compose family No. 46, the "Sarcostomes" or "Proboscidés." The names are in French: Bibion, Syrphe, Stratyome, etc. The generic names are repeated in the tabular outline of the family on page 452, again in the French vernacular, with brief key characters. On page 446 appears the entry of present interest: "Tétanocère, les mouches nommées par Fabricius, ***nigripennis***, ***marginata***, ***reticulata***, ***vesicularia***, ***elata***, ***clavata***, ***planifrons***, ***furcata***, ***palustrata***, ***cucularia***, etc."

This reference in Millin's Magasin is the one usually given for Duméril's "Exposition." The article and that in the Journal de physique (see item 3) appear to be almost identical, differing only slightly in the word order of the title, in the different pagination because of the size difference between the two journals, and in slightly different word order in introducing lists of species (cf. Tétanocère).

(5 and 6) 1801.—Duméril, "Plan d'une méthode naturelle pour l'étude et la classification des Insectes," Bull. Soc. philomatique 2: 153–155 (reviewed in detail, 1802, in Wiedemann's Archiv für Zoologie und Zootomie 3, pt. 1: 202–209). A brief note, essentially an abstract of the "Exposition," outlining his method of classification. No Diptera are mentioned by name. Only the tabular key to orders and the key to one family of beetles are used.

(7) 1802.—Latreille, "Histoire naturelle, gén. et part., Crust. et Ins.," vol. 3. On page 460, "XI. Tétanocères; ***tetanocerae***. (Duméril)," as one of the groups under "Division seconde" of the genus ***Musca***, and including numerous species of rather diverse habitus. All of these group names under ***Musca*** are given in the plural, with such names as ***curvipennes***, ***longipedes***, ***carnariae***, etc.

(8) 1804.—Latreille, "Nouveau Dictionnaire d'Hist. Nat.," vol. 24, Tableau Méthodique, p. 196. "Tétanocère, ***Tetanocera***," with description and one included species, ***Musca graminum*** Fabricius. Duméril's name is not mentioned. This is the earliest valid use of the name ***Tetanocera*** in Latin or Latinized form and as a "substantive in the nominative singular." The following genus on the same page is the related ***Dictya*** Meigen (1803), with three Fabrician species, ***Musca reticulata***, ***M. cucullaria***, and ***M. umbrarum***.

(9) 1804, 1807.—Duméril, "Traité élémentaire d'histoire naturelle," first and second editions. I have been able to see only the second edition (1807). All generic names are used in the vernacular (*e.g.*, tétanocère, p. 41), and the generalized and elementary nature of the discussion makes it unlikely that the first edition would reveal any difference in the use of the names.

(10) 1805.—Latreille, "Histoire naturelle, etc.," vol. 14, p. 385. "***Tetanocera*** (Duméril)," with two Fabrician species included, ***Musca reticulata*** and ***M. planifrons***. The preceding genus (p. 384) is ***Dictya***, with a footnote stating that the names ***Dictya*** and ***Tetanocera*** had been transposed in the Nouveau Dictionnaire.[2]

(11) 1806.—Duméril, "Zoologie analytique." Only vernacular names (*e.g.*, ***Tétanocère***, p. 283) are used in the tabular keys, but the

[2]In passing, it is significant to note that whether ***Tetanocera*** is dated from 1804 or 1805, not one of the originally included species in either case is ***Tetanocera*** in the current sense.

text has both Latinized and vernacular, as for example, "Les *tétanocères* (*tetanocerus*)" (p. 282). No species are mentioned.

Duméril (1823, p. 264), after reviewing his first "tentatives" in Cuvier (1800), his manuscript classification, the extracts, and his elementary treatise of 1804, states that "Cependant ce n'est réellement qu'en l'année 1805 que mon travail sur les insectes parut en entier dans la *Zoologie analytique* . . . ," with 62 synoptic tables.

(12) 1809.—Latreille, "Genera Crust. et Ins., vol. 4, p. 350. "*Tetanocera, Tétanocère*," with "*Tetanocera* Dum., Latr." and "*Dictya* Latr., Meigenii" cited in synonymy. Many species cited by name.

(13) 1810.—Latreille, "Considérations générales, etc." On page 404, "Tétanocère. *Tetanocera*," with brief description. On page 444, "Tétanocère. *Scatophaga marginata*, Fab.; ejusd. *dictya umbrarum, oscinis planifrons*." There is no valid type designation here, for the species which seems to stand by itself as a designated type (*marginata*) is after all not one of the species originally included by Latreille (1804).

(14) 1817.—Latreille, *in* Cuvier's "Le Règne Animal," vol. 3, p. 645: "Les Tétanocères. (*Tetanocera* Dum.)." No species mentioned.

(15) 1823.—Duméril, "Considérations générales sur la classe des insectes." Page 230, the generic diagnosis is headed by "Tétanocère; *Tetanocerus*, Dum. (pl. 49, fig. 3)." No species are mentioned on this page. The legend for figure 3 is "Tétanocère réticulé," in which one can recognize the species *reticulata* Fabricius.

Chapter VIII (pp. 240–266) is entitled "De la Bibliographie Entomologique, . . . ," and in this Duméril discusses the publications of 56 authors, including himself (pp. 264–265). He mentions his work in Cuvier (1800) as his "premières tentatives," his manuscript on classification presented to the Société philomatique, the extracts ("Exposition"), and his later publications (1804, etc.), but nowhere does he mention a 1798 paper.

(16) 1860.—Duméril, "Entomologie analytique," Mém. Acad. Sci., Institut Impérial de France, vol. 31, parts 1 and 2. Page 1255: "Genre Tétanocère. *Tetanocera*. (Duméril)," and he states that he published the genus under this name in 1800 and again in 1806 (actually as Tétanocère and *Tetanocerus*, respectively!).

In Part 1, Preface, p. v, is this footnote: "Mes premiéres publications sur l'histoire naturelle des insectes datent de 1797. ***Exposition d'une méthode naturelle pour l'étude et la classification des insectes.*** Magasin encyclopédique de Millin, an IV, tome I, page 289,—et de 1799." He goes on from here to note the "tableau général" inserted in Cuvier's "Anatomie comparée."

The juxtaposition of the date 1797 with the title of the "Exposition" and the citation in Millin's Magasin, might lend credence to the claim that such a paper by Duméril was indeed published about 1797 or 1798. Some confusion certainly exists here, however. I have examined a complete set of Millin's Magasin from its first appearance in 1795 (Année 1, tome 1) through the VI. Année, tome 4 (1800), when the "Exposition" did actually appear, and I can find no earlier article by Duméril on classification. Furthermore, his own reference is undeniably incorrect: If "an IV" is interpreted as the fourth year of the

French Revolution, that is 1795–1796 and Duméril had no papers at all in that year in Millin's Magasin; on the other hand, if "an IV" is interpreted as meaning Année IV or the fourth series, the paper in that series, volume 1, page 289, is by Du Mont Courset on the effects of light on plants. Duméril did publish a paper in 1797 on the natural history of insects ("Dissertation sur l'organe de l'odorat, etc.," Millin's Magasin, 3 Année, tome 2, pp. 435–446), and this appears in bibliographies as his first published work on insects. One can only conclude that the footnote of his 1860 book is either an error, a misinterpretation by Duméril himself of the citation in a reprint of his paper (as did Osten Sacken, 1900, q.v.), or a misleading juxtaposition of a reference to his 1797 paper on natural history with the title of the "Exposition," and the latter in addition with an incorrect reference.

(17) 1860.—Lucas, "Liste des Travaux d'Entomologie publiés de 1795 à 1860, par M. C. Duméril," Ann. Soc. Ent. France (ser. 3) 8: 653–662. The reference for the "Exposition" is cited as "Magas. encycl.," tome 4, p. 433, an VI (1798)." This reference repeats a common error, as is easily demonstrated by trying to find it in the Magasin for an VI (1798) rather than in Année VI (=sixth series) for an VIII. See earlier discussion under Duméril, 1800.

(18) 1900.—Osten Sacken, "Notiz über die Erstlingsarbeit von C. Duméril über entomologische Classification, mit besonderer Rücksicht auf die Gattung *Tetanocera*," Verhandl. Zool.-Bot. Gesell. Wien, 50: 450–451.

Osten Sacken calls attention to two different separates of Duméril's "Exposition" in his possession, the one quarto, the other "Klein-Octav," the latter with a statement that it was a separate with special title and pagination from the "Magasin Encyclopédique, vol. IV, p. 433, an VI (1798)." Osten Sacken points out slight differences in wording between the two separates, under *Tetanocera* (cf. earlier discussion under Duméril, 1800).

Unfortunately, Osten Sacken did not see the originals, and he concluded that "*Tetanocera*" apparently must date from 1798. From the sizes given for the reprints, and from the quoted wordings under *Tetanocera*, it is clear that Osten Sacken's quarto copy was a reprint from the Journal de physique, the octavo from Millin's Magasin.

Another point that might be raised is that the originals in these journals used only the vernacular form "Tétanocère," but the reprints might have used the Latinized form. I do not know whether the reprints also used the vernacular spelling, and Osten Sacken misquoted them, or whether they actually used the spelling *Tetanocera*, and if so, on what date the separates were actually issued. At any rate, I am convinced that the 1798 reference is wrong, and that the separates were based on the 1800 originals in the two journals. It seems extremely unlikely that the names would have been changed to the Latin form merely for the separates.

CONCLUSIONS

1. There is no publication by Duméril (1798) on classification.

2. The generic name *Tetanocera*, under the International Rules of Zoological Nomenclature, technically dates from Latreille (1804),

where it is monobasic with ***Musca graminum*** Fabricius as genotype. Earlier publications used only a vernacular form.

3. ***Tetanocerus***, while possibly the spelling which Duméril intended in the first place, officially dates from Duméril (1806).

The zoological consequences of these bibliographical researches, and what should or might be done about them, are outside the province of this paper, and are best dealt with by authors specializing in the families and genera concerned. Enough is known to indicate that the history of ***Tetanocera*** and its relation to ***Dictya*** Meigen (1803) and several later names and their included species and genotype designations form a difficult and complex problem that would have been greatly simplified had the above facts been clarified long ago.

THE COLLEMBOLA OF THE STATE OF NEW YORK, by ELLIOT A. MAYNARD. xxii+339 pages, frontispiece, 37 plates (669 figures), 4 text figures. Comstock Publishing Company, Ithaca, New York, 1951. Price, $7.50.

A comprehensive study, such as the present work, of the Collembola of an important geographical area in the United States is an outstanding contribution to our knowledge of this relatively poorly studied order. The reasons for the neglect of the taxonomy of the Collembola are rather obvious: these insects are not commonly seen by the ordinary observer; they do not have strikingly interesting habits; they are of relatively little economic importance; and, like larval specimens they must be kept in liquid preservatives or as whole mounts on slides and, consequently, do not make attractive cabinet specimens. Only two important works have previously been published on this order as it occurs in the United States, Guthrie's *Collembola of Minnesota* (1903) and Mill's *Monograph of the Collembola of Iowa* (1934).

Dr. Maynard's monograph seems to have been quite thoroughly done. It is significant that it has been in course of preparation for more than twenty years. Keys are given throughout for all taxonomic categories; these are of a simple dichotomous type and are mechanically well constructed, the characters used being apparently sufficient for the separation of the categories involved. Descriptions are brief but to all appearances, diagnostic. Both structural characters and entire insects are shown in the illustrations, five plates of which (including the frontispiece) are in color. Considerable ecological information is included in the discussions of the individual species. Finally, a bibliography, apparently in the true sense of the word, of the Collembola of the world, which is appended, should be an invaluable aid to future students of the order.

The book presents a very good appearance, and has few typographical errors. The illustrations appear to be well executed. The book should have wide usage even outside its geographical area, even more so than usual because of the wide distribution of many of the Collembola.—M. T. J.

A STUDY OF CERTAIN METABOLIC INTERMEDIATES IN THE NORMAL AND DDT-POISONED HOUSE FLY ADULT[1]

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Although the existence and indispensable role of certain metabolic intermediates in animal tissues has become well established, very few of these investigations have been carried out using insect tissue. For example, insects were omitted from the classic invertebrate studies of Eggleton and Eggleton (1928), Meyerhof and Lohman (1928a, b), Meyerhof (1928), and Needham *et al.* (1932). Interest in the wing muscles of flying insects, unparalleled in the animal kingdom for their high rate of contraction and relaxation, led Baldwin and Needham (1933) to study the phosphorous distribution in the thoracic muscles of adult *Calliphora* and *Lucilia* flies. Before this work was finished, a paper by Schütze (1932) appeared in which he reported having examined insect muscles of *Locusta*, *Dytiscus*, *Hydrophilus*, *Lucanus*, *Apis* and *Aeschna* and found arginine phosphate to be present, relying however, only on the evidence of the rate of hydrolysis. He also analyzed for lactic acid. Heller (1936) studied the phosphorous compounds in the larva and adult of the sphingid hawkmoth, *Deilephila euphorbiae* (Linn.).

The phenomenal success of DDT, 2, 2-bis(p-chlorophenyl)-1, 1, 1-trichloroethane, as an insecticide has led Merrill *et al.* (1946) and Ludwig (1946) to investigate the physiological and biochemical changes in insects poisoned with DDT with the hope of explaining the toxicity of this insecticide. More recently, Barron and Tahmisian (1948), Gilmour (1948), and Humphrey and Siggins (1949) have investigated various aspects of muscle metabolism in insects. This investigation is concerned with the analysis of certain metabolic intermediates in the house fly, *Musca domestica* L., under both normal conditions and those conditions brought about by poisoning with DDT.

EXPERIMENTAL

Methods

Rearing and Handling the Insects.—The common house fly, which is a conveniently reared laboratory insect possessing a known high susceptibility to DDT, was used in this investigation; rearing was done according to the standard procedure described in the Soap Blue Book (1948). From 15 to 20 grams of the fly pupae were placed in small screen-wire rearing cages and the adults allowed to emerge. The adult flies were fed for five days on a diet of a 10 percent sugar and

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water solution before they were used. By avoiding the use of milk as food for the adult flies, more nearly constant values for phosphorus were obtained. To facilitate handling the adult flies at the time of making the analyses, they were transferred from the rearing cages by a rapidly moving air stream to one-quart cardboard cylindrical cartons provided with screened ends. These cartons were then placed in a battery jar into which a stream of carbon dioxide was passed continuously, thus utilizing the rapid and reversible anesthetic effect of carbon dioxide (Williams, 1946). The immobilized flies were transferred from cardboard cartons to glass-stoppered 40 x 50 mm. weighing bottles and the total weight of the flies was determined. Following this weighing process, all the flies were placed in a normal atmosphere where they recovered completely from the anesthesia. The use of carbon dioxide was adopted only after it was established that it had no effect upon the metabolic intermediates being studied.

Each day's rearing of flies was divided into three approximately equal lots. Lot A, consisting of untreated normal flies, was analyzed immediately after weighing and chilling. Lot B, consisting of untreated normal flies, was transferred, while still immobilized by the carbon dioxide to a one-quart cardboard carton and then placed in the rearing room where it remained for six hours without food or water. Lot C, consisting of DDT-treated flies, was transferred, while still immobilized by the carbon dioxide to a one-quart cardboard carton which contained a glass plate coated with DDT crystals at the rate of 100 milligrams of DDT per square foot. The flies of Lot C also were placed in the rearing room where they remained for six hours. The flies in Lot C responded to the toxic effects of the DDT within 15 to 30 minutes and for the duration of the six-hour period were found on the bottom of the carton manifesting various symptoms of DDT poisoning. The six-hour period was found to produce the optimum response to the DDT-poisoning without causing death to any of the insects. At the end of six hours, the flies of Lots B and C were treated with carbon dioxide and transferred to the weighing bottles and quickly weighed. Losses in weight during the six-hour period were recorded and the flies were then frozen preparatory to analysis.

The Distribution Method.—The distribution method described by Umbreit *et al.* (1945), involving fractionation of a trichloroacetic acid tissue extract, was adapted to this study of certain phosphorylated intermediates in both the normal and DDT-poisoned house fly adults. The method, as applied to the adult house flies, consisted of an intermittent trichloroacetic acid extraction of the "acid soluble" phosphorous in the ground whole insects. An aliquot of this extract was set aside for analysis and the remainder was separated by utilizing the solubilities of the constituents in the extract in the presence of barium ions and ethyl alcohol. It was possible to study quantitatively the following components present in the trichloroacetic acid extract of the normal and DDT-poisoned whole insects: total phosphorous, inorganic phosphorous, "true" or calcium precipitable inorganic phosphorous, arginine (by difference and by determination of arginine), pyruvic acid, reducing substances as glucose equivalents, and non-protein nitrogen. In addition to these components, the major portion of the

original trichloroacetic acid extract was separated into three fractions: a barium insoluble fraction (I), on which analyses were carried out for total phosphorous and fructose; a barium soluble, alcohol precipitable fraction (II), on which analyses were carried out for total phosphorous, inorganic phosphorous, "true" or calcium precipitable inorganic phosphorous, arginine phosphate (by difference and by determination of arginine) and reducing substances as glucose equivalents; and a barium soluble, alcohol soluble fraction (III), on which analyses were carried out for total phosphorous. In addition, analyses were made of the tissue residue for phosphorous; and the water content and water loss in normal and DDT-poisoned flies were recorded.

Homogenate Extraction.—The homogenate extraction process consisted of grinding lots of whole, frozen house fly adults, varying in weight from two to eight grams (approximately 100 to 400 flies), depending upon the type of analysis being made, in a Pyrex glass homogenizer patterned after the original model introduced by Potter and Elvehjem (1936). Approximately 10 milliliters of ice-cold 10 per cent trichloroacetic acid solution were used for each one gram of flies. The tissue homogenizer and its contents were kept immersed in crushed ice during the grinding process. The grinding process was completed usually in three to five minutes and the homogenizer tube with its contents were transferred to a centrifuge and the tissue residue separated from the extracting solution. The centrifugate was filtered quickly by suction into a 125 milliliter filtering flash immersed in crushed ice. One or two additional extractions were performed on the tissue residue remaining in the homogenizer tube, using 10- to 15-milliliter portions of cold five percent trichloroacetic acid solution, and centrifuging and filtering as before. The combined, filtered trichloroacetic acid extracts possessed a clear, bright yellow color; the tissue residue was retained for total phosphorous determination.

The Fractionation Process.—The volume of the trichloroacetic acid filtrate was recorded and a 10-milliliter sample was removed, neutralized and transferred to a 50 milliliter volumetric flask where it was diluted to final volume with distilled water and used for the analyses listed under distribution method above. The remainder of the original extract, which was kept chilled in crushed ice, was adjusted to pH 8.2 with sodium hydroxide, using phenolphthalein as an indicator. This titration was quite important because variations of 0.5 pH unit would cause the barium insoluble fraction to become contaminated with compounds which would otherwise occur elsewhere. Therefore, the titration was followed with a Beckman pH meter in addition to using the indicator solution since the normal yellow color of the extract made it difficult to establish the pink-colored end-point. A slight excess of barium ion, in the form of barium acetate, was added to the titrated extract; 0.05 of a milliliter of a 25 percent barium acetate solution was added for each milligram of phosphorous theoretically present. After the addition of the barium acetate, procedure "A" of Umbreit *et al.* (1945) was followed to produce the fractions I, II, and III described above. The entire extraction and fractionation process required approximately six hours, during which all operations were carried out at a temperature of zero degrees centigrade. Specific

analyses were begun as soon as possible after completing the fractionation process.

Analytical Methods.—Colorimetric micro-methods were employed for the quantitative determination of the various metabolic intermediates using the Evelyn (1936) macro-colorimeter. Standardization curves were prepared by using the data obtained from the pure compounds in each case. All the methods followed Beer's law over the range used and did not appear to fluctuate appreciably as evidenced by checks with the standard solutions. Each series of analyses included a standard which agreed with the standard curve within the limits of precision of the method.

Phosphorous.—The basic method for the determination of phosphorous, developed by Fiske and Subbarow (1925) was used for the determination of inorganic phosphorous including phosphoarginine, "true" inorganic or calcium precipitable phosphorous, total phosphorous in the trichloroacetic acid extract, and the tissue residue phosphorous in the extracted tissue. The inorganic phosphorous, including phosphoarginine, was determined on a sample taken from both the original trichloroacetic acid extract and fraction II. In contrast to phosphocreatine, which is decomposed rapidly by the molybdate reagent, phosphoarginine is decomposed with more difficulty. Meyerhof (1928) found the maximum rate of hydrolysis of both the pure arginine phosphoric acid and also of the trichloroacetic acid extract of crab muscle occurred at a 0.01 N concentration of hydrogen ion. Therefore, in order to insure the complete hydrolysis of the phosphoarginine, the sample for this determination was diluted in a 50 milliliter volumetric flask with sufficient 10 N sulfuric acid and distilled water to approximate a 0.01 N concentration of hydrogen ion. This diluted sample was allowed to incubate overnight at a temperature of 28° to 30° C. The "true" inorganic or calcium precipitable phosphorous was determined by the method given by Umbreit *et al.* (1945) which is adapted from that of Fiske and Subbarow (1929). The total phosphorous was determined by the method given by Umbreit *et al.* (1945). The tissue residue phosphorous was determined by quantitatively transferring the tissue residue which remained from the trichloroacetic acid extraction to a porcelain evaporating dish, adding from 15 to 30 grams of a 2 :1, sodium carbonate : potassium nitrate fusion mixture and slowly evaporating to dryness. The mixture was heated further until fusion was complete, as indicated by the mixture turning grayish-white. Upon cooling, the mixture was dissolved in distilled water, the solution was transferred quantitatively to a volumetric flask and total phosphorous determined as for inorganic phosphorous.

Pyruvic Acid.—The pyruvic acid content of the protein-free extract was determined by method "B" of Friedemann and Haugen (1943), who further modified the 2, 4-dinitrophenylhydrazine method of Case (1932) which is based on the characteristic of pyruvic acid to form a hydrazone. For all practical purposes, the 10 percent trichloroacetic acid sample (to which had been added iodoacetic acid, titrated with sodium hydroxide to pH 7.8, to a final concentration of 0.2 per cent) contained only two keto acids, pyruvic and α-ketoglutaric, the method "B" proved to be highly specific for pyruvic acid. The pyruvic acid

standard was prepared by using the procedure reported by Robertson (1942) for the preparation of the sodium salt.

Phosphoarginine.—Two methods were used for the estimation of phosphoarginine in a portion of both the original trichloroacetic acid extract and fraction II. The first method involved the determination of the phosphorous difference between the inorganic plus phosphoarginine phosphorous and the "true" inorganic phosphorous as obtained by calcium precipitation. The second method involved the quantitative determination of arginine by the Sims (1945) modification of the Sakaguchi reaction.

Fructose.—Roe's (1934) modification of the Seliwanoff reaction was used for the determination of fructose in the diluted original extract and in fraction I.

Glucose.—The micro blood sugar method developed by Folin and Malmros (1929), and more recently modified and adapted to the photoelectric colorimeter by Horvath and Knehr (1941), was used for the determination of reducing substances, as glucose equivalents, present in the diluted original extract and in fraction II.

Nitrogen.—The micro-chemical, colorimetric method used for the determination of total nitrogen was taken from Umbreit *et al.* (1945) as modified from the method of Johnson (1941).

Results

The analytical data presented in Table I summarize certain metabolic intermediates and related compounds as found in lots of whole, ground normal and DDT-poisoned house fly adults. All data are calculated on the basis of the original wet weight of the tissue. The average and standard deviation values are based on not less than ten sets of data for each experimental condition, except for the pyruvic acid values which are based on seven. Those data which do not list standard deviations represent average values based on a minimum of at least three determinations for each experimental condition except for the values for phosphorous recovered which represent the sum of the average values reported for total phosphorous for fractions I, II, and III. Analyses for fructose were carried out on fraction I with fructose-diphosphate should occur. The values obtained were so low as to be unreliable. However, analyses for fructose on the original extract gave a good qualitative test.

In the process of weighing lots of flies for analysis and comparing the weight loss of those flies which had been untreated and unfed for a period of six hours with the weight loss of those flies which had undergone DDT tremors for six hours, it became apparent that a significant additional weight loss occurred in the DDT-poisoned flies. Therefore, the experimental conditions used in preparing flies for analysis were duplicated on 13 lots of flies taken from the same rearing cages as the flies used in the analyses, but in this case the flies were killed by heat and dried for several days at 110° C. to a constant weight. In this manner, data were obtained which indicated the initial weight of the flies, as taken from the rearing cages, consisted of an average water content of 73.5 percent. The average water content of untreated flies, after remaining in the rearing room for six hours without food or water, was 76.8 percent of their original net weight. And the average water

content of the DDT-treated flies which also had remained in the rearing room for six hours without food or water was 78.4 percent of their original wet weight. The small but consistent difference, average 1.6 percent, that occurred between the flies of the two latter experimental conditions suggested an increased weight loss due to greater catabolic

TABLE I

SUMMARY OF CERTAIN PHOSPHORYLATED METABOLIC INTERMEDIATES AND RELATED COMPOUNDS AS FOUND IN HOMOGENIZED AGGREGATES OF WHOLE NORMAL AND DDT-POISONED HOUSE FLY ADULTS*

ANALYSES	EXPERIMENTAL TREATMENT†		
	A	B	C
	mg. %	mg. %	mg. %
Original trichloroacetic acid extract:			
Total phosphorous	140 ± 11	144 ± 9	155 ± 11
Phosphoarginine by difference	392 ± 83	175 ± 27	121 ± 15
Phosphoarginine by arginine determination	113 ± 11	87 ± 15	69 ± 9
Pyruvic acid	8.4 ± 1.8	4.7 ± 1.5	4.1 ± 0.6
Reducing substances as glucose equivalents	300	287	147
Non-protein nitrogen	314	285	221
Fraction I:			
Total phosphorous	39 ± 21	35 ± 11	37 ± 15
Fraction II:			
Total phosphorous	85 ± 15	91 ± 17	109 ± 11
Phosphoarginine by difference	93 ± 31	47 ± 12	38 ± 18
Phosphoarginine by arginine determination	40 ± 3	32 ± 5	24 ± 4
Reducing substances as glucose equivalents	51	51	38
Fraction III:			
Total phosphorous	3.8 ± 1	5.1 ± 0.6	6.7 ± 0.8
Phosphorous recovered: sum of total phosphorous found in fractions I, II and III	128	131	153
Tissue residue:			
Total phosphorous	135 ± 14	140 ± 11	155 ± 10

*All data calculated on the basis of original wet weight of tissue.

†Experimental Treatment of Flies: A, normal flies, analyzed as taken from the rearing cage; B, normal flies, held six hours without food or water; C, DDT-Poisoned flies, held six hours without food or water.

activity in the DDT-treated flies. The difference in weight loss between the untreated and DDT-treated flies after six hours was more striking. The untreated flies lost an average of 14.4 percent of their original wet weight during the six hour period while the DDT-treated flies lost an average of 24.6 percent of their original wet weight during the

same period; the difference between these two conditions being 10.2 percent.

DISCUSSION

In this work it was necessary to establish values for the normal insect. In so doing, it was recognized that what information exists relative to intermediary metabolities in insects is derived largely from analyses of the whole insect or portions of the insect body. By this means only the grossest alterations can be detected and if the breakdown of a given substance in one part of the body is compensated by its synthesis in another, analysis of the whole insect may show no change. The average total phosphorous in the trichoroacetic acid extract of the whole normal housefly, 140 ± 11 percent, compared favorably with previously reported values for other insects (Schütze 1932, Baldwin and Needham 1933, and Heller 1936). The small differences in total phosphorous in the original extract and the three fractions did not reflect any significant changes due to the effects of DDT.

Some difficulty was encountered in obtaining consistent values for the total phosphorous of fractions I and II. Baldwin and Needham (1933) encountered the same difficulty and attributed the inconsistency to the interfering effect of the relatively large amount of hexose ester occurring in fly muscle which makes up a large part of the fly's thorax. They precipitated with barium at pH 7.4 – 7.8; as they point out, the disadvantage of this procedure is that the inorganic phosphate is not completely precipitated at this pH, and to obtain the true preformed inorganic phosphate, a separate estimation is necessary. In this work, precipitation was carried out at pH 8.2; although Umbreit *et al.* (1945) indicate that 0.5 of a pH unit deviation is allowable, it is possible that a more critical adjustment of the pH should be made in the case of tissues containing a high amount of hexose esters.

The high values of phosphoarginine obtained by the method of difference on the original extract are misleading since they probably represent the phosphorous obtained from the hydrolysis of other phosphorylated intermediates; these values do agree, however, with those of Baldwin and Needham (1933), who also obtained their values by the method of difference.

SUMMARY

A distribution method was employed for studying some of the phosphorylated metabolic intermediates occurring in homogenized aggregates of whole normal and DDT-poisoned house fly adults. Values for phosphorous, phosphoarginine (by phosphorous difference and by arginine determination), pyruvic acid, reducing substances as glucose equivalents, and non-protein nitrogen were determined for (A) normal flies, (B) normal files held six hours without food or water, and (C) DDT-poisoned flies held six hours without food or water (See Table I). Considerable and highly significant differences were found between (A) normal flies and (B) normal flies held six hours without food and water.

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INSECT COMMUNITIES OF STANDING DEAD PINE (PINUS VIRGINIANA MILL.)[1]

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In the succession from an old field to an oak-hickory climax forest on the inner edge of the Coastal Plain of Maryland, the Virginia pine (*Pinus virginiana* Mill.) is the first tree invader. It reaches maturity, dies, and is replaced by hardwood stands.

The species of insects inhabiting these dying and dead pine trees have been covered in part by works dealing with the economic species. Hopkins (1901, 1902, 1909, 1911, and 1915) has published on bark beetles and other Coleoptera which damage the living as well as the dying and dead trees used for timber. Beal and Massey (1945) published a comprehensive paper on the Scolytidae of North Carolina and listed the associated parasites and predators. The biology and habits of some species of wood inhabiting insects have been published by Doane, Van Dyke, Chamberlin and Burke (1936) and Balduf (1935 and 1939).

There have been some very interesting papers on fallen trees as ecological units. Shelford (1913) stated that there was a "consocies of logs" in which there is a regular succession of forms. Standing dead trees are included in his "tree stratum." The ecology of a number of the important forms found in white pine (*Pinus strobus* L.) logs was discussed by Graham (1925) with data on temperature and moisture relations. Perhaps the most extensive work published on the species inhabiting cut pine and oak logs was that done by Savely (1939) in North Carolina. Blackman and Stage (1924) published an extensive paper on the succession of insects in hickory.

Despite all the work that has been done, there is a conspicuous absence of information on the ecology of standing dead trees, which are more the rule than the exception in pine stands undergoing invasion by deciduous trees. Also, little has been published concerning the species existing on the outside surface of the pines, which, during the summer at least, compose in many respects a fairly stable community.

This report deals with the insect associations of standing dead pine trees and includes information on the tree-surface fauna.

Observations were made at College Park, Maryland, in a well-drained 10-acre tract of second growth forest adjacent to the campus of the University of Maryland at an altitude of 80 to 120 feet. The forest was composed principally of white oak (*Quercus alba* L.) along

[1]The research upon which this paper is based was done while the authors were at the University of Maryland, Department of Entomology.

with smaller numbers of red oak (*Quercus borealis* Michx. f.) and black oak (*Quercus velutina* Lam.), both young and old trees being represented. Numerous young hickories (*Carya tomentosa* (Lam.) Nutt.), some dogwood (*Cornus florida* L.), a few red maples (*Acer rubrum* L.), and a few young beeches (*Fagus grandifolia* Ehrh.) composed the remainder of the deciduous trees. A conspicuous element was the mature (approximately 70 to 80 years old) *Pinus virginiana* Mill. which in number of large trees ranked next to the oaks. These trees undoubtedly were a carry-over from the almost pure stand of Virginia pine that preceded establishment of the deciduous trees. It was with the dying and dead members of these lingering pines that this investigation was concerned.

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The majority of the specimens collected are in the authors' collections. The remainder are in the U. S. National Museum.

METHODS

Trees were observed during day light at least once weekly over a 12 month period beginning in February, 1948. Occasional observations were made by the junior author during 1939–42. Only dead standing trees were considered in this study. In many cases, particularly with the pines in advanced stages of decay, the trees were leaning, held from the ground by near-by trees. In many cases the tops of the trees had been broken off and only a 10 or 15 foot section remained standing. Those with trunks resting on, or within 4 feet of the ground, were not considered standing. The approximate length of time that a tree had been dead was estimated by its condition as described below. Quite a number of the trees studied were known to have died during or just previous to the 1939–42 period of observation so there is no question about how long they had been dead.

Stage 1. *Dying to one-half year dead:* Trees with needles green to red-brown, bark firm and difficult to remove, and with scolytid pitch tubes abundant. Sixteen trees were studied.

Stage 2. *One-half to one year dead:* Trees with dead needles largely unshed, with bark beginning to loosen, and with the sap almost dry. Twelve trees at this stage were studied.

Stage 3. *One to two years dead:* Trees with few needles still on the twigs and with the bark unbroken but moderately loose. Almost all

of the scolytids had disappeared, but a number of forms associated with them were still present. Eleven trees were studied.

Stage 4. *Two to four years dead:* Trees with all needles fallen and with bark loose and exfoliating above the 15 or 20 foot level. Hibernating insects were common under the looser bark. Fourteen trees were studied.

Stage 5. *Four to six years dead:* Trees with bark remaining only on the lower 15 or 20 feet of the trunk and very loose, but frequently unbroken. In the damper places under the bark, fungi were in evidence. Many hibernating forms were present. Twelve trees were studied.

Stage 6. *Six to eight years dead:* Trees with bark almost entirely fallen off and with many of the larger branches broken off. The sapwood of all was punky and riddled with termite galleries. Fungi were evident in all the damp portions of these trees, of which 10 were studied.

Stage 7. *Eight to ten years dead:* Trees with only the trunk left standing; the heartwood was still firm, but termites could be found in it. Saprovorous forms prevalent. Seven such trees were studied.

Stage 8. *Ten years dead and over:* Four such trunks broken off at levels of about 10 to 15 feet were found. Trees with the sapwood reduced almost to pulp, and with only a small hard core of heartwood remaining. In every instance, the tree was very damp with saprovores as the major forms present.

DISCUSSION

During the course of the work, 184 species of insects and allied forms were collected from a total of 86 dying and dead pine trees. Figure 1 shows the number of species present in different parts of the trees.

Fifty-nine species were fairly numerous during the summer on the outside surface of the pines. (About 47 of these were confined to trees of stages 1, 2 and 3.) Of this total one cannot disregard 40 species that were consistently present and represented true pine-inhabiting species. The remaining 19 species were considered to have occurred incidentally with no obligate relation to the pine or its associated community. The Araneae in particular were present in large numbers only in the summer and then the majority were on the surface of the tree and not under the bark, at least during the daytime when these observations were carried out. In winter, these forms were found under the bark in some instances, but only for hibernation, and then they did not visibly affect the community there. Other forms that occurred consistently and continually during spring and early summer were the adult buprestids and a number of cerambycids. Scolytids were found on the outside of the trees only rarely. The larvae of many surface species were an important part of the communities found under the bark, in the sapwood, and in the heartwood of the dead standing trees.

Figure 1 shows how the number of possibly incidental species diminishes as one goes deeper into the tree, and also that the largest number of species occur under the bark and in the sapwood. Probably this

is because the greatest amount of available nutrients occurs in these portions (Savely 1939). The least number of species (17) was found in the heart wood. The list of species from under the bark and, to some extent, from the sapwood is augmented by a considerable number of hibernating species. Savely (1939) states that only advanced larval stages of some of the large forms can utilize the small amount of nutrients present in the heartwood, and this perhaps explains the small number of species found in this region.

The effect of available nutrients may also be illustrated in figure 2, where the total number of species found in each stage of dead pine is

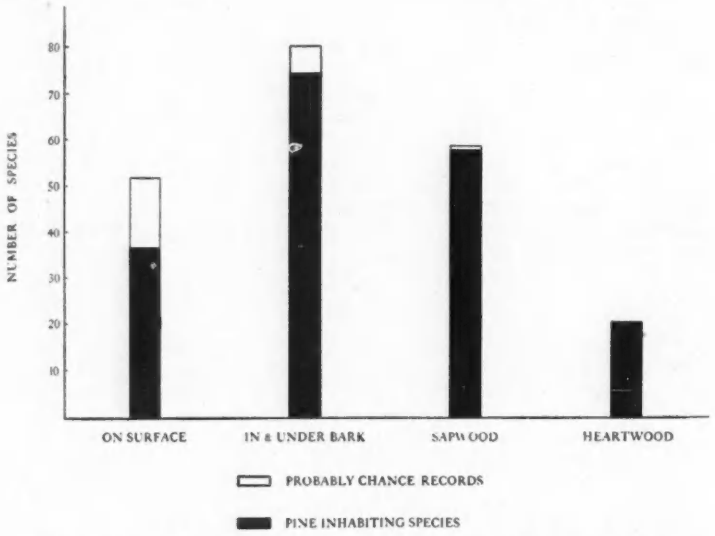

FIG. 1. Numbers of species present in different parts of the tree. Based upon 86 dead standing pines in all stages of decomposition.

listed. It seems that as the period since death increases and nutrients for the wood-boring forms diminish, the number of species decreases. The increase of species in the fifth stage might be accounted for by an overlapping of communities when there are still a number of wood-boring forms remaining and the saprovores have begun to appear. Fifth stage trees are the last bearing any appreciable amount of bark under which a number of forms can hibernate. In stages 6, 7, and 8, the sapwood has become semi-pulpy, in most cases riddled with insect burrows, and is thoroughly permeated by fungi. The most numerous forms are saprovores and general feeders, with tenebrionids being a conspicuous element.

The contrast between the complex scolytid, cerambycid, and buprestid community found in stages 1, 2, and 3, and the few forms

found in stages, 4, 5, 6, 7, and 8 is shown in figures 3 and 4. Figure 3 shows the general interrelationship of the commoner species in the fresh dead pines. The scolytids not only are the primary invaders, often being the cause of death to the tree, or at least hastening it, but they are by far the most numerous single group. Their number was usually on the decline by the time buprestid and cerambycid larvae were noted. However, it was due to the scolytids that the majority of species was present, either as predators or otherwise. Figure 4 shows the relatively few forms that were commonly present in stages 4 through 8. Some of the species were present in all later stages, this being particularly true of the termites.

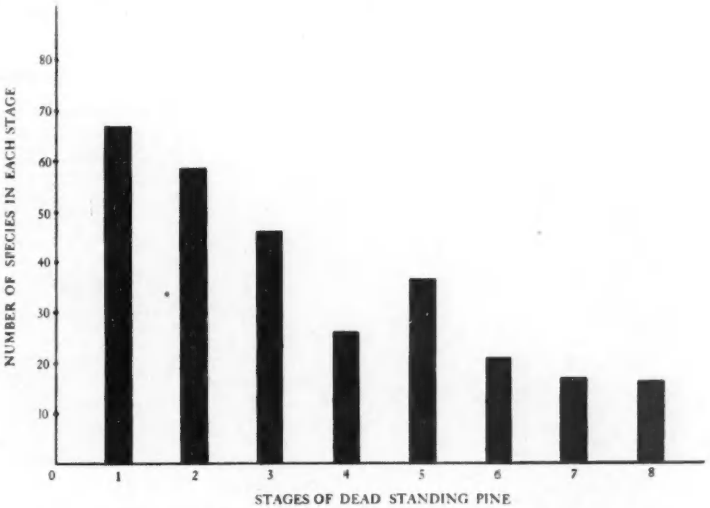

FIG. 2. Total number of species found in each stage of dead standing pine. (For number referring to stage of pine, see section on *Methods*.)

In figure 4 the stage of tree in which a certain species was particularly common is usually noted numerically after its name. In this figure it can be seen that in stages 4 and 5 wood-feeding insects were still slightly more numerous than the other forms. Also, in these stages there were only two common saprovorous species noted. The major group present, at least numerically, was the termites and around them the other species form a loose community.

In stages 6, 7, and 8 there is a better defined community, based on the prevalent saprovorous forms. There were numerous colonies of ants present, but they were concentrated usually in the nest and did not seem to affect the other forms present to any great extent. In no instance were any annelid worms noted as has been reported by Savely

(1939) for felled logs; this is apparently because of insufficient moisture. No nematodes were seen.

Only a few vertebrates were observed. They were: downy woodpeckers, ***Dryobates pubescens***, Northern fence swifts, ***Sceloporus undulatus hyacinthinus*** (Green), and several grey tree frogs, ***Hyla versicolor versicolor*** (LeConte). Salamanders, commonly recorded in fallen log communities, were not found.

In conclusion it might be stated that many of the insects present in stages 1, 2, and 3 are common only to pines, while many of the insects found in the later stages (5, 6, 7, and 8) are found in almost every tree in the woods (oak, maple, etc.) in an advanced stage of decay.

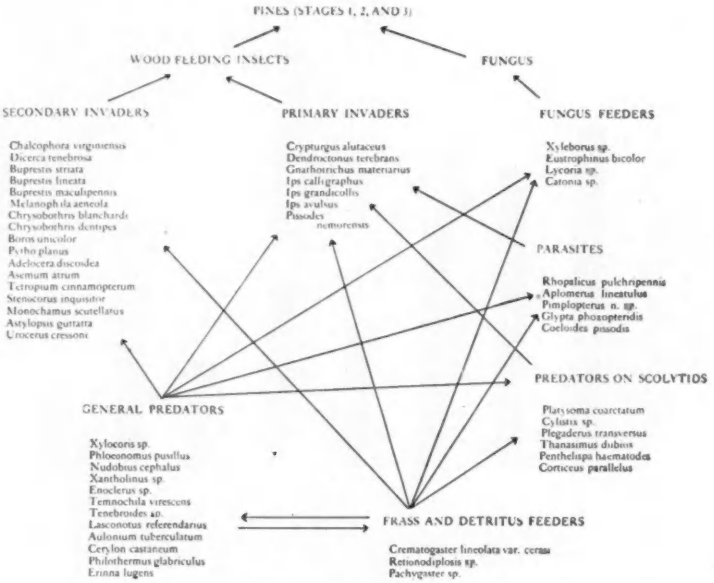

FIG. 3. Generalized food web of some of the insects inhabiting the early stages of dead standing pine.

SUMMARY

One hundred and eighty-four species of insects and related forms were found in 86 dead standing pines (***Pinus virginiana*** Mill.) Observations were made at least once a week between February, 1948, and March, 1949.

Recently dead trees supported a numerically greater number of species than did trees over 2 years dead. Apparently this was due to the nutritional value of the fresh dead standing pines being higher than in the older trees (figure 2).

After being dead 6 or more years, there were relatively few trees that remained standing, but in the few trees examined (21) there seemed to be a fairly constant relationship of forms.

The community of recently dead trees was centered around the scolytids, while after the first 6 months the buprestids and cerambycids became the dominant forms. Several years later in the same trees termites became the most numerous forms, while still later they were partially replaced by the saprovores.

The communities found in the standing dead pines differed remarkably in some groups from those recorded by other authors from downed logs.

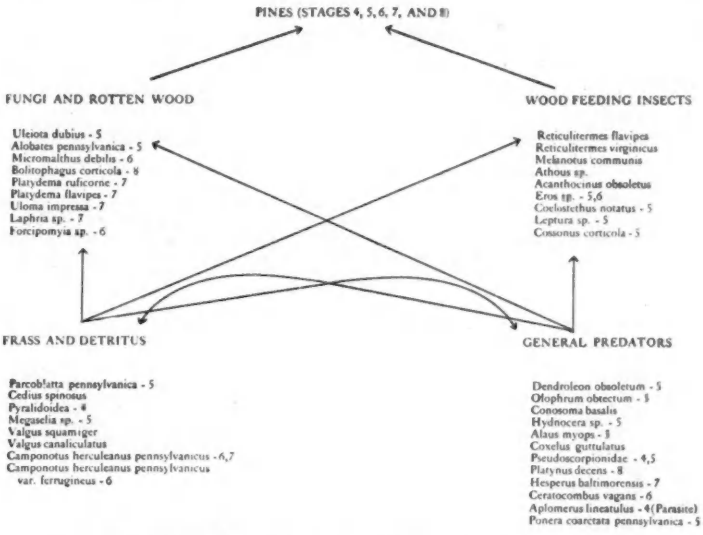

FIG. 4. Generalized food web of some of the insects inhabiting the later stages of dead standing pine.

The spring and summer community on the ***outside*** of the standing pines is composed largely of characteristic forms, at least in trees dead only 1 year (figure 1).

The largest number of forms in stages 1, 2 and 3 was found just under the bark. This number gradually decreased to almost a total absence of forms in the heartwood of these trees. This was probably due to the fact that the nutrient value is reportedly greatest just under the bark and in the sapwood (figure 1).

ANNOTATED LIST

General statements referring to habits such as omnivorous, predatory on insects and parasitic were taken from statements in Balduf (1935, 1939), Brues (1946), Chamberlin (1939), Clausen (1940),

Keen (1938), Knull (1946), Muma (1943), and other works previously mentioned. Blatchley (1910, 1926) was used mainly for insect identification and not for habits. Such statements as under bark, ovipositing and feeding on a specific species are the authors' own observations.

The list of Coleoptera follows the order in Leng (1920, 1927), Leng and Mutchler (1933), Blackwelder (1939, and Blackwelder and Blackwelder (1948). All other insects are listed in the order found in Brimley (1938).

The following abbreviations are used: # = stage of pine; 1/I/48 = date of collection.

ORTHOPTERA

Blattidae

Parcoblatta pennsylvanica (DeGeer). #2, 3, and 4. Under bark. All year. Omnivorous.

Gryllidae

Oecanthus exclamationis (Davis). #4. In top. 23/VII/48. Chance occurance.

Orocharis saltator (Uhler). #1. In top. August, 1948. Generally arboreal.

ISOPTERA

Termitidae

Reticulitermes flavipes (Kollar). #1 through 8. Galleries in bark and wood. All year except during very cold weather. Wood feeding.

Reticulitermes virginicus (Banks). #2 through 8. Galleries in bark and wood. All year except during very cold weather. Wood feeding.

NEUROPTERA

Myrmeleonidae

Dendroleon obsoletum (Say). #5. Larva. Under bark in termite gallery. 1/V/48.

HEMIPTERA

Pentatomidae

Brochymena carolinensis (Westw.). #3, 4, and 8. Under bark. 30/IX/40, III/48. Hibernating, but a species associated with living pine.

Brochymena quadripustulata (Fab.). #2. Under bark. March. Hibernating.

Euschistus servus (Say). #8. Under loose wood. 21/III/48. Hibernating.

Tingitidae

1 specimen. #2. On trunk. 20/III/48. Chance occurrence.

Reduviidae

Melanolestes picipes (H-S). #8. In base. 6/IV/48. Hibernating and insect predatory.

Rocconota annulicornis (Stal.). #1. In top. 13/VII/48. Insect predatory.

Anthocoridae

Xylocoris sp. #1. Nymphs. Under bark. II through VI/48. Insect predatory. Feeding on *Lasconotus referendarius* Zimm.

Cryptostemmatidae

Ceratocombus vagans (McA-M.). #6. In decayed sapwood VI/48. Predatory on mites.

HOMOPTERA

Fulgoridae

Catonia spp. #2, 3, and 5. Nymphs. Under bark. II and III/48.

COLEOPTERA

Carabidae

Tachys proximus (Say). #2 and 3. Under damp bark and decayed wood. All year. Predatory on insects and mites.

Platynus decens Say. #8. Decayed wood. 8/IV/48. Predatory on insects and mites.

Calleida viridipennis (Say). #3 to 5. Under bark. Winter 1939 and 40, II/49. Predatory.

Plochionus timidus Hald. #5. Under bark. 8/IV/48, 14/IV/49. Predatory.

Pinacodera limbata (Dej.). #5. Under bark. Winter 1939 and 40.

Staphylinidae
Phloeonomus pusillus Grav. #1. Under bark. III/48. Predatory on insects.
Olophrum obtectum Er. #5. In wood. V/48. Predatory on insects.
Nudobius cephalus (Say). #1 and 2. Under bark. X and XII/47, III and VI/48. Predatory on insects; found in Scolytidae galleries.
Xantholinus sp. #2. Larvae. Under bark. 1/VII/48. Predatory on insects.
Hesperus baltimorensis (Grav.). #7. Under bark. V/48. Predatory on insects.
Conosoma basalis (Er.) #3 and 5. Under bark. III and V/48. Predatory.
Aleocharinae. #1 and 2. Under bark. Winter and spring. Predatory on insects.
Pselaphidae
Cedius spinosus Lec. #2, 3, and 4. Associated with termites. II through spring.
Scaphidiidae
Scaphisoma convexum Say. #6. In decayed wood. 18/II/49. Probably hibernating.
Histeridae
Platysoma coarctatum Lec. #1. Under bark. III through V/48. Predatory on scolytids.
Cylistix sp. #1. Under bark. Spring/48. Predatory on bark beetles.
Plegaderus transversus Say. #1 and 2. In scolytid galleries. III through spring and fall. Predatory.
Genus (?). Larvae. #4. Under bark. 16/III/48.
Lycidae
Eros trilineatus Melsh. #1. On trunk. 3/VII/48. Predatory.
Eros sp. #7. Larva. Damp wood. 20/III/48. Predatory.
Lampyridae
Lucidota corrusca (L.). #3. On trunk. III/48. A species common on tree trunks of all types during winter.
Cleridae
Thanasimus dubius (Fab.). #1. On bare trunk. VII/48. Predacious on bark beetles.
Enoclerus sp. #1 and 2. Larvae. Under bark. III/48. Predatory.
Hydnocera sp. #5. On trunk. 12/VIII/48. Predatory.
Hydnocera sp. #2. Larvae. Under bark. II/48.
Micromalthidae
Micromalthus debilis Lec. #6. Larvae. Damp wood. 25/III/48. Rotten wood-feeding.
Pythidae
Pytho planus Hbst. #2 and 4. Under bark. 7/III/42 and 20/III/48.
Pytho planus Hbst. #2. Larvae. In wood. II and VII/48. Feeding on bark and cambium.
Elateridae
Adelocera discoidea (Web.). #3, 4, and 5. Under bark. II and III/48. Hibernating. Female on #1 ovipositing.
Adelocera discoidea (Web.). Larvae. #2. Under bark. II, III, VI/48. Feeding on decaying wood.
Alaus myops (Fab.). #2 and 5. Under bark. II and III/48. Hibernating.
Alaus myops (Fab.). #2 and 3. Larvae. In wood. II and III/48. Predatory on insects.
Athous sp. #3, 6, and 8. Larvae. In wood. III/48.
Elater xanthomus Germ. #3, 5, and 6. In wood and under bark. 4/II/39, 7/III/42 and 16/III/48.
Elater xanthomus Germ. #2, 3, 6, and 7. Larvae. Under bark. II, III, VII/48. Probably predacious.
Melanotus communis (Gyll.). #3 to 8. II through spring and 23/VII/48. Under loose bark. Hibernating.
Melanotus communis (Gyll.). #6 and 8. Larvae. In wood. III/48. Feeding on rotten wood.
Melasidae
Dromaeolus sp. #2. Larvae. Under bark. 21/II/48.
Throscidae
Aulonothroscus sp. #2 and 3. Under bark. III/48 and VII/48. Chance.

Buprestidae
Chalcophora virginiensis (Drury). #1. On trunk. V and VI/48.
Chalcophora virginiensis (Drury). #3 and 7. Larvae. In wood. XI/48 and 20/III/48. Feeding on wood.
Dicerca tenebrosa (Kby.). #1. On bare trunk. 23/VI/48. Ovipositing.
Buprestis striata Fab. #1. On trunk. V and VI/48. Ovipositing. #2, 5, and 6. In sapwood. In winter 48. Before emergence.
Buprestis lineata Fab. #1. On trunks. VI through VII/48. Ovipositing and copulating.
Buprestis maculipennis Gory. #1. On trunks. VI to VII/48. Ovipositing.
Buprestis consularis Gory. #1. On trunk. 11/VI/49. Ovipositing.
Melanophila aeneola Melsh. #1. On branch. 6/VII/48. Wood feeding as larvae.
Anthaxia quercata (Fab.). #1. On trunk. 23/VI/48. Chance occurrence.
Chrysobothris blanchardi Horn. #1. On trunk. 29/VI/48. Wood feeding as larvae.
Chrysobothris dentipes (Germ.). #1. On trunk. VI and VII/48. Wood feeding as larvae.
Chrysobothris sp. #2 and 5. Larvae. Sapwood. II and III/48. Wood feeding.

Ostomidae
Temnochila virescens (Fab.). #1 and 2. Larvae. Under bark. II and III/48. Predatory.
Tenebroides sp. #2. Under bark. IV/48. Predacious.

Nitidulidae
Meligethes mutatus Harold. #1 and 2. Spring. Chance.
Epuraea sp. #1. Under bark. 16/III/48. Chance hibernation.
Glischrochilus sanguinolentus (Oliv.). #3. Under bark. 8/V/39.

Rhizophagidae
Rhizophagus bipunctatus (Say). #2. Under bark. 3/I/49. Hibernating..
Rhizophagus cylindricus Lec. #1. Under bark. 1/I/39 and 17/II/40.

Cucujidae
Silvanus bidentatus (Fab.). #3. Under bark. IV/48.
Catogenus rufus (Fab.). #5. Under bark. 8/IV/48. Also #2. In wood. 24/V/48. Parasitic on cerambycid larvae.
Cucujus clavipes Fab. #3. Under loose bark. 20/XI/48.
Uleiota dubius (Fab.). #3 to 6. Under bark. All year.
Uleiota dubius (Fab.). #5. Larva. In loose wood. 3/VII/48.

Colydiidae
Coxelus guttulatus Lec. #2 to 8. Under bark in spring and fall. In loose wood in summer.
Lasconotus reflexendarius Zimm. #1 to 3. In scolytid galleries and under loose bark on upper portion of trunk. IV/48 to V/48. Predatory.
Aulonium tuberculatum Lec. #2. In scolytid galleries. II/48 and 17/II/40.
Penthelispa haematodes (Fab.). #1 to 3. In scolytid galleries. 4/II/39, spring and fall 1948.
Cerylon castaneum Say. #2 and 3. Under bark. All year, especially spring.
Philothermus glabriculus Lec. #1 to 4. Under bark. 4/II/39, spring and fall 48.

Mycetaeidae
Rhanis unicolor (Ziegl.). #3. Under bark. III/48. Hibernating. Fungus feeding.

Endomychidae
Aphorista vittata (Fab.). #8. In dry wood. 8/IV/48. Hibernating.

Alleculidae
Hymenorus sp. #3. Larvae. In sapwood. 25/III/48.

Tenebrionidae
Bolitophagus corticola Say. #8. Adults and larvae. In decaying wood. Spring and fall.
Diaperis maculata Oliv. #8. In rotten wood. 4/II/39, 4/I/41. Fungivorous.
Platydema ruficorne Sturm. #4 and 8. Under loose bark and rotten wood. III/48.
Platydema flavipes (Fab.) #3 to 8. Under damp bark and in rotten wood. III and IV/48.

Platydema ellipticum (Fab.). #3. In wood near fungi. 18/IV/48.
Uloma impressa Melsh. #7. In rotten wood. III, IV/48.
Corticeus parallelus (Melsh.). #1 and 2. In scolytid galleries. 7/I/39 and all year, 48, most numerous in winter. Larvae with adults in spring.
Alobates pennsylvanica (DeG.). #4 and 5. Under loose bark. I and II/48. Hibernating.
Alobates pennsylvanica (DeG.). #5. Larvae. In wood. III/48.
Boros unicolor Say. #2 and 3. Under bark. II/39 and III/48.
Boros unicolor Say. #2 and 4. Larvae. In cambium. Spring, 1948.

Melandryidae
Eustrophinus bicolor (Fab.). #3. Under bark. III and IV/48. Saprovorous.

Anobiidae
Coelostethus notatus (Say). #5. Adults and larvae. Feeding in dry hard wood. 16/III/48.

Cisidae
Genus (?). #2. Under bark. 16/II/48.

Scarabaeidae
Cotinis nitida (Linn.). #1. On trunk. 10/VIII/48. Chance occurrence.
Valgus squamiger (Beauv.). #7 and 8. With termites. Spring/48. Inquiline.
Valgus canaliculatus (Fab.). #7 and 8. With termites. Spring/48. Inquiline.

Cerambycidae
Asemum atrum Esch. #1. On trunks. IV/48. Wood feeding.
Asemum atrum Esch. #2 and 3. Larvae. In phloem and sapwood. II and III/48.
Tetropium cinnamopterum Kby. #1 and 2. Larvae. Under bark. III/48. Wood feeding.
Stenocorus inquisitor Linn. #2, 3, and 4. In cells under bark. Winter/48. #1. On trunks. IV/48.
Stenocorus inquisitor Linn. #1 to 4. Larvae. In bark and sapwood. III/48.
Leptura sp. #2. Larvae. In wood. 21/II/48. #5. In wood. 1/VII/48.
Typocerus zebra (Oliv.). #5. On trunk. 5/VI/48.
Typocerus zebra (Oliv.). #6. Larvae. In wood. 25/III/48.
Monochamus scutellatus (Say). #1. On trunks in shade. IV/48 and 49. Some ovipositing.
Monochamus scutellatus (Say). #1 and 2. Larvae. In sapwood and heartwood. Summer/48.
Leptostylus sp. #2 and 3. Larvae. In wood. II through VII/48.
Astylopsis guttata (Say). #1 and 2. In tops. VI to VIII/48.
Acanthocinus obsoletus (Oliv.). #2 and 8. Larvae. In phloem. III/48.

Chrysomelidae
Cryptocephalus notatus Fab. #2. On bark. 24/V/48. Chance.
Paria canella (Fab.) #2 and 5. Under bark. III/48. Chance hibernation.
Chalcoides flulvicornis (Fab.) #2. On bark. 5/VI/48. Chance.
Mantura floridana Cr. #7. In decaying wood. 28/III/48. Chance hibernation.
Dibolia borealis Chev. #2. Under bark. 16/II/48. Chance hibernation.

Mylabridae
Bruchus sp. #2. Interstices of bark. III and IV/48. Chance hibernation.

Curculionidae
Pissodes nemorensis Germ. #1 and 2. On trunks. IV/48.
Pissodes nemorensis Germ. #2 and 3. Larvae. In wood. III to fall/48. Wood feeding.
Cossonus corticola Say. #2 and 7. Larvae and adults. Under bark and in wood. All year.
Rhyncolus discors Csy. #5 and 6. Under bark and in wood. III, IV/48.

Scolytidae
Crypturgus alutaceus Schwarz. #1. Under bark on upper trunk. 1/VII/48.
Dendroctonus terebrans (Oliv.). #1. Under bark at base of trunk. 16/III/48. Adults and larvae. #1 and 2. Under bark at and below soil level. XII/48.
Gnathotrichus materiarius (Fitch). #1. Adults and larvae. Under bark and in sapwood. III and fall/48.
Ips calligraphus (Germ.). #1. Adults and larvae. Under bark. 1/39, II/40 and spring and fall/48.

Ips grandicollis (Eich.). #1 and 2. Adults and larvae. Under heavy bark. I/39, II/40 and all year 1948.
Ips avulsus (Eich.). #1 and 2. Under thin bark on upper trunk and branches. Spring/48.
Xyleborus sp. #2. In radial galleries in sapwood. I/39 and III/48.

LEPIDOPTERA

Phalaenidae
Genus (?). #6. Pupa. Under loose bark. III/48.
Pyralidoidea
Genus (?). #4. Larvae. Under bark. 16/III/48.
Gelechiidae
Genus (?). #8. In rotten wood. III and IV/48. Chance occurrence?

DIPTERA

Itonididae
Retinodiplosis sp. #1, 2, and 3. Larvae. Under bark. II and III/48.
Rhagionidae
Erinna lugens (Loew.). #2. Larvae. Under damp bark. II through VII/48.
Stratiomyiidae
Pachygaster sp. #2. Larvae. Under bark. II/48.
Asilidae
Atomosia puella (Wied.). #1. On trunks. VII and VIII/48. Chance occurrence?
Cerotainia macrocera (Say). #1. On trunks. VII/48. Chance.
Laphria sp. #7. Larvae. In rotten wood. 20/III/48. Predatory.
Bombyliidae
Spogostylum oedipus (Fab.). #1. On trunk. 2/VII/48. Chance occurrence?
Dolichopodidae
Medeterus sp. #1. On trunks. VII/48.
Medeterus sp. #1 and 2. Larvae. Under bark. II throughout summer/48.
Phoridae
Megaselia sp. #5. Larvae. Under bark. III/48.
Larvaevoridae
Winthemia rufopicta (Reinh.). #1. In spider's web and on trunks. 29/IX/48. Chance occurrence.
Winthemia sp. #3. Pupa. Under bark. 25/III/48. Chance occurrence.
Sarcophagidae
Sarcophaga sp. #1. On trunk. 13/VII/48. Chance occurrence.
Muscidae
Muscina assimilis (Fall). #3. Under bark. III/48. Hibernating.
Helina procedens (Walk.). #3 and 4. On trunks. VII/48.
Pyrellia cyanicolor (Zett.). #5. Under bark. 17/III/48. Hibernating.
Drosophilidae
Chymomyza amoena (Loew.). #1. On trunk. 10/VII/48. Probably chance occurrence.
Milichiidae
Desmometopa sp. #2 and 3. Larvae. Under damp bark. II and III/48.
Fungivoridae
Lycoria sp. #2. Larvae. Under bark. 21/II/48.
Heleidae
Forcipomyia sp. #6. Larvae. In moist sapwood. 25/III/48.

HYMENOPTERA

Siricidae
Urocerus cressoni (Nort.). #1. On trunk. 29/IX/48. Ovipositing.
Diprionidae
Genus (?). #5. Pre-pupae. Under loose bark. 3/VII/48. Pupating.
Braconidae
Coeloides pissodis (Ashm.). #1. On trunks. VI to VIII/48. Parasitic on *Pissodes* spp.
Coeloides pissodis (Ashm.). #2. Cocoon. Under bark. II/48.
Iphiaulax agrili (Ashm.). #1. On trunks. VI/48. Chance occurrence.

Ichneumonidae
Aplomerus lineatulus (Say). #4. In cerambycid burrow. 1/VII/48.
Pimplopterus n. sp. #3. On trunk. 11/VIII/48.
Glypta phoxopteridis (Weed). #1. On trunks. VII/48. Some ovipositing.
Chalcidae
Rhopalicus pulchripennis (Crfd.). #1. On trunk. VII/48. Parasitic on scolytids.
Eurytomidae
Eurytoma tomici (Ashmd.). #1. Under bark in scolytid galleries. II and III/48. Parasitic on scolytids.
Eupelmidae
Eupelmus sp. #1. On trunks in shade. VII/48. Chance occurrence.
Formicidae
Ponera coarctata pennsylvanica (Buckley). #5. On trunks and under bark. 16/III/48 and VII/48.
Crematogaster lineolata (Say). #5. Under bark. VII/48.
Crematogaster lineolata var. *cerasi* (Fitch). #2. Under bark. 21/II/48.
Aphaenogaster fulva aquia (Buckley). #5. Adults and pupae. In wood. VII/48.
Aphaenogaster tennesseensis (Mayr.). #5. In termite galleries. 1/VII/48. Carrying off worker termites.
Strumigenys pulchella (Emery). #2. Under bark. 21/III/48.
Camponotus nearcticus (Emery). #8. In sapwood and heartwood. III/48.
Camponotus herculeanus pennsylvanicus (DeG.). #6 and 7. In wood. All year. Insectivorous.
Camponotus herculeanus pennsylvanicus var. *ferrugineus* (Fab.). #6. In wood. 25/III/48.
Chrysididae
Chrysis sp. #3. In wood. 11/VII/48.
Mutillidae
Sphaerophthalma pennsylvanica (Lep.). #5. On trunk. 12/VIII/48.
Bethylidae
Cephalonomia sp. #1. On trunk. 30/VII/48. With larva of *Synchita* sp. in mandibles.
Vespidae
Genus (?). #3. Larvae. Under bark. III/48.
Halictidae
Augochlora pura (Say). #7. In wood. 6/V/48.

CLASS ARACHNIDA

ARANEAE

Micryphantidae
Ceraticelus alticeps (Fox). #1. Found on twigs. 30/VII/48.
Pisauridae
Dolomedes sp. #1. Young. In tops. VIII/48.
Pisaurina mira (Walck). #1. Under loose bark. 29/IX/48.
Oxyopidae
Oxyopes scalaris (Htz.). #1. On twigs. 30/VII/48.
Theridiidae
Europis funebris (Htz.). #1. In top. 13/VIII/48.
Theridion lyra (Htz.). #1. On trunk. 30/VII/48.
Theridion murarium (Em.). #1. Adult and young. In top. 13/VIII/48.
Argiopidae
Neoscona arabesca (Walck). #1. On trunk. 13/VIII/48.
Thomisidae
Coriachene versicolor (Keys). #1. On trunk. 13/VIII/48. #3. Under bark. 25/III/48.
Philodromus sp. #2. Under bark. 21/II/48.
Clubionidae
Clubiona pallens (Hentz). #1. Young. In top. 13/VIII/48.
Salticidae
Metaphidippus sp. #1. Young. In top. 13/VIII/48.
Hentzia sp. #5. Young. On trunk. 30/VIII/48.

Marpissa undata (DeG.). #1. Under loose bark. 29/IX/48.
Phidippus putnamii (Peckham). #1. On twigs. 30/VII/48.
Phidippus whitmanii (Peckham). #1. On trunk. 2/VII/48. Young and juveniles. VIII/48.

Segestriidae
Ariadna bicolor (Htz.). #5. On trunk. 3/VII/48.

PSEUDOSCORPIONIDA

Pseudoscorpionidae
Genus (?). #2, 3, 4. Under loose bark. II throughout spring/48. Insect predatory, observed feeding on termites.

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DECIDUOUS FORESTS OF EASTERN NORTH AMERICA, by E. LUCY BRAUN. xiv+596 pp. Blakiston Company, Philadelphia, 1950. Price, $10.00.

All the eastern deciduous forests of the United States have been divided into three parts, according to most accounts of them, ever since their first exploration by American plant ecologists. This three-part conception, associated especially with Clements, has been recognized for some time as an unnatural one. In Dr. Braun's new book a more realistic picture is presented, one in which the traditional beech-maple, oak-hickory, and oak-chestnut associations are put in their places as parts of a larger and far more complex vegetational pattern, divided into nine forest regions of the eastern United States north of peninsular Florida. To the discussion of these and of the pattern as a whole the author has brought a life-work of study of the eastern forests, and the book which results is the best treatment available for any of the world's major plant formations.

A brief statement of some ecological principles is given first. The theoretical position outlined is on the whole a conservative one, but differs importantly from tradition in American ecology by regarding the associations, or major parts of the forest formation, as abstract and artificial, and not real, units. The largest part of the book is then devoted to descriptions of the forest regions, each being described in sufficient detail that the account is a valuable reference on the vegetation of any part of the eastern forests in which a naturalist might be working and a guide to further vegetational literature. The author suggests that the forest regions may be natural entities with observable boundaries, and that in the study of plant and animal distribution, ranges of species be given in terms of these regions, rather than of states. While the diversity of species distributions should no more be expected to fit simply and naturally into forest regions than into life-zones or other such man-made geographic divisions, the forest regions are clearly improvements over states as biological areas. The last part of the book discusses the history of the eastern forests, drawing together information from paleobotany, existing distributional disjuncts and relicts, and glacial and physiographic history to interpret the evolution of the forests to their present pattern. Development of the eastern forests from the widespread, mixed forests of the Tertiary, often called the Arctotertiary Forest, is traced; and the central relation of the present mixed mesophytic forests of the Southern Appalachians (where the author's own work played a major part in changing our conception of the eastern forests) to the whole pattern is indicated. This historical section, the most significant part of the book, should be a useful reference for any zoologist working with distributional or phylogenetic problems of animals in the eastern United States. The relation may be illustrated by Braun's emphasis of the Southern Appalachians as a geographic center for the deciduous forest formation, as they have long been known to be (since Adams, 1902) for some groups of animals.

Deciduous Forests of Eastern North America can be recommended as a thorough and scholarly, and probably in major outline definitive, treatment. More suitable as a reference work than for reading, it offers the entomologist the best account to be had of the vegetation of some areas with which he may be dealing and of the vegetational history of the eastern forests which is part of the background of the history of their fauna.—R. H. WHITTAKER.

CONSTRUCTION OF THE SCALE COVERING OF AONIDIELLA AURANTII (MASK.)[1]

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The California red scale, *Aonidiella aurantii* (Mask.), is an armored scale which, like most of this group, protects itself with a covering that is not a part of the insect body. This covering is made of material secreted by glands in the insect's body, most of them in the pygidium. The cast skins from the molts are incorporated into the covering, as are bits of loose bark and debris from the surface of the plant. The covering completely encloses the insect, although the ventral portion, between the insect and the plant surface, is usually very thin. The dorsal covering of the California red scale is thin in comparison with most other scale coverings. It is translucent enough that the yellow-orange color of the insect's body shows through. This suggested the name "red scale." The covering is sufficiently thick and tough, however, to protect the insect very effectively.

This paper describes the method the California red scale uses in constructing its covering. It also gives a limited amount of information on the composition of the covering.

LIFE HISTORY

The California red scale is ovoviviparous. The newly born insect or "crawler" has eyes, antennae, and legs, and walks about until it discovers a suitable place to settle. It then settles down, tucks its legs and antennae beneath its body, and inserts its stylets into the host plant. Waxy filaments secreted from the dorsal surface become felted together, and the insect passes into the whitecap stage. The body becomes progressively more flattened and enlarged, and is shield-shaped by the time of the first molt. The legs are lost at this molt.

After the first molt, the molted skin or exuviae is incorporated into the scale covering, which is then extended outward around the periphery of the exuviae in such a way that it maintains a roughly circular form. As soon as the covering has been extended enough to accommodate the full size of the second instar, construction ceases. The insect body then grows until it almost fills the covering and becomes attached to it, and the insect molts for the second time. After the second molt the cover is again extended, this time to its final size; the body grows to its final size and shape and again becomes attached to the covering.

Thus, the order of growth for the second and third instar of this insect is as follows: (1) the insect molts; (2) the covering is extended

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The author wishes to express appreciation for advice and assistance given by Mrs. Metta McD. Johnson and Dr. D. L. Lindgren.

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to the full size required for that instar; and (3) the insect body grows until it almost fills the covering and becomes attached to it. A slight amount of growth occurs during the production of the covering, but most of the growth takes place immediately after the covering is completed.

While forming its covering, this insect is turbinate, or similar in shape to the mature San Jose scale. After the second molt and the completion of the covering in its final form, the prosoma grows back and almost around the pygidium, so that the insect takes on the characteristic circular form found in the genus *Aonidiella*.

CONSTRUCTION OF COVERING

The covering of the California red scale is so thin and semitransparent that it is possible to look through it and watch the insect at work forming it. Observations made in this way are recorded below.

The covering is formed from material secreted as filaments by the glands in the pygidium. These rather large glands, of which there are almost fifty, discharge through ducts that open on the dorsal surface or at the edge of the pygidium. There are also about forty very small glands whose ducts open on the ventral surface.

The material used in making the covering is extruded from these glands in the form of soft cylinders and is formed into the covering by the actions of the insect itself. The insect uses its pygidium much as a plasterer uses a trowel, in this case a self-feeding trowel that continually produces its own plaster. The insect is very active during this period and moves itself around rotatively, and in and out, anteriorly-posteriorly.

The pygidium is moved in a number of ways. The tip of the pygidium is lifted, and the lobes and plates on its posterior edge are rubbed against the under surface of the scale covering. These movements usually occur in groups of three, one along a mid-line and one, each, to right and left. The pygidium is arched to rub the under surface of the covering with its smooth dorsal surface, which contains the duct openings. These movements are not continuous; groups of five or six rapid movements are usually separated by one to several minutes of quiescence. The pygidium is also moved slowly over the under surface of the covering, in contact with it and adhering to it, depositing covering material as it is secreted.

The edge of the covering is extended by pressing the tip of the pygidium against an area at the edge and slowly shoving outward, building a flatly triangular extension the size and shape of the pygidium. The pygidium is then drawn back, the body rotates. the pygidium is re-applied, and another portion of the edge of the covering extended. While extending the covering, the tip edge of the pygidium is held down tight to the surface of the substratum, so that it scrapes up cuticle wax and incorporates it into the covering, together with any bits of dirt, loose bark, or other substances on the surface. I have never seen the pygidium extend beyond the covering.

Though the scale covering is extended discontinuously, an extension of the edge in one place often being followed by deposition of material on the older part of the covering before the covering is extended in another sector, the insect manages to keep the edge of the covering

relatively even and practically equidistant from the exuviae in all sectors. Any slight break in the cover is repaired. One insect was observed which had suffered a slight accident, about a quarter of the scale covering having been pressed down flat on the fruit approximately halfway from the edge. This insect spent the nine hours that it was under observation moving back and forth along this pressed-down section, raising it up again.

It has been known for some time that the California red scale must rotate while secreting its covering, since, although most of the material

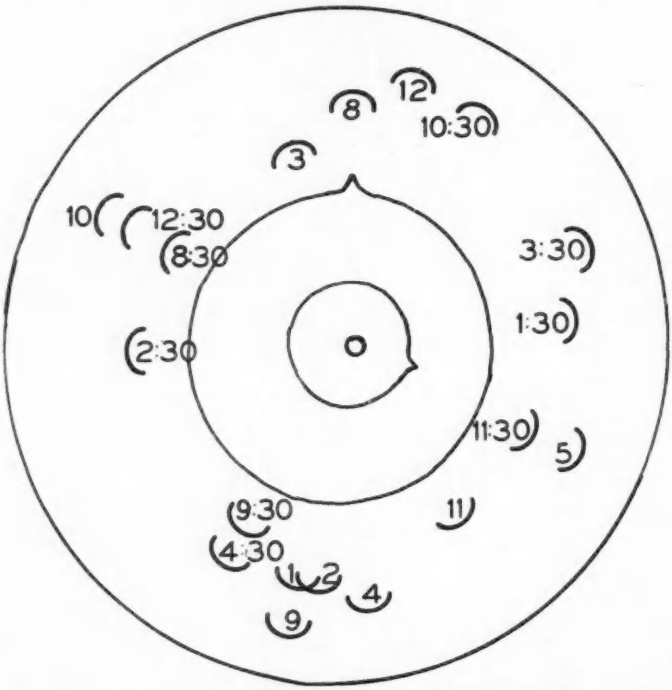

FIG. 1. Positions of the pygidium of a third instar California red scale while forming its covering, shown at half-hour intervals from 8 a. m. to 5 p. m. Clock time is indicated. Scale covering is shown diagrammatically. Exuviae and nipple are indicated.

used is secreted by the large glands of the pygidium, the covering is circular. Furthermore, the pygidial tips of the two molted skins may point in different directions, neither one paralleling the pygidium of the mature insect. It also appeared probable that the movement could not all be in one direction, since the stylets are inserted and the insect is feeding actively while the covering is being formed. Nel (1933) made some observations of the insect while depositing its covering and reported a "rotating" motion.

The observations reported below were made of female red scales which had laid down one-third to three-quarters of the last covering, the older scales being at about the stage when fertilization ordinarily occurs. Certain insects were selected, marked, and observed at intervals of 30 minutes throughout the day. Others were observed continuously for short periods of not more than 2 hours.

Figure 1 shows the positions of the pygidium of one specimen at half-hour intervals from 8 a. m. to 5 p. m. one day. During the nine-hour period, it made almost four complete revolutions. Figure 2 shows 24 positions of another specimen through a two-hour period.

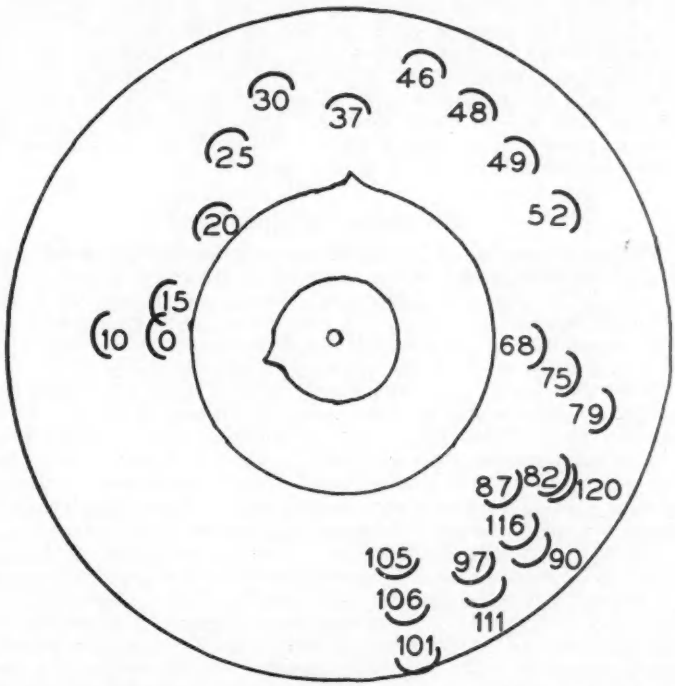

FIG. 2. Twenty-four successive positions of the pygidium of a California red scale while forming its covering. Figures indicate minutes elapsed after the first observation.

While depositing the covering, insects were observed to rotate through as much as 540 degrees, or one and one-half times a full circle. An insect would rotate in one direction, say clockwise, usually for about a full circle, but sometimes more or less; then it would rotate in the other direction approximately the same distance. This rotation was not a simple movement, but was combined with in-and-out (anterior-posterior) movements. The combination of the two produces an irregu-

lar circling motion, so that the line traced by the tip of the pygidium is an eccentric circular line extending around the second exuvia, at times touching the edge of the exuvia and at times the edge of the covering. Thus, in the formation of the covering, any given point on it is covered many times. At 76° F., the time required to complete a circle, ranged from 2 to 3½ hours. The rate of movement was not constant, the insect sometimes remaining practically motionless for 5 minutes or more. The rotating movement is usually imperceptible and can be detected only after a lapse of 15 or 20 seconds, and then only in relation to a fixed point.

One result of the random method of deposition is that the covering is much thinner at its edge than at any other part, since this last portion to be formed has not been gone over so many times and therefore has not had so much material deposited on it. The young break out through this portion soon after the first of them are born. According to Ebeling (1936), spray oil penetrates at this thin-edge portion of the covering. It seems that the oil must penetrate through the edge rather than under it, since the covering is tightly sealed to the surface, usually under a certain amount of cuticle or loose bark.

COMPOSITION OF COVERING

The scale covering of *Aonidiella aurantii* has been referred to as "waxy," or as composed of wax secreted by the insect, together with the exuviae or cast skins. However, Metcalf and Hockenyos (1930) extracted the scale coverings of a number of other species with hot carbon tetrachloride and reported that waxes accounted for only 31 to 58 percent of the weight of the coverings of various species.

After the discovery by Bartlett and Martin (1945) that cocoons of the potato tuberworm, *Gnorimoschema operculella* (Zell.), could be dissolved by a dilute (1.3 percent) solution of sodium hypochlorite, D. L. Lindgren tried the same solution on the California red scale. The sodium hypochlorite solution broke down the structure of the scale covering, leaving an unorganized whitish mass. This suggested that a part of the scale covering is composed of a material similar to silk.

The top coverings of 3,000 female red scales grown on washed grapefruits were removed just as the coverings were completed and before the insect bodies had become attached to them. These coverings were dried and weighed. They were then extracted with benzene in a Soxhlet extractor for several days to remove the waxes or other benzene-soluble materials. The extracted coverings and the waxes were dried and weighed separately. The coverings were then treated with hot 10 percent NaOH solution for three minutes to break down the secreted protein-type material, but leaving the exuviae, which were filtered out, washed, dried, and weighed. Average weights per scale cover are shown in Table I. The original weight of the scale covering (18.1 micrograms) is about 0.85 percent of the total weight of a mature female red scale as given by Lindgren and Sinclair (1944). Our figures indicate that the covering of a California red scale is about 8 percent exuviae, 45 percent waxes, and 47 percent proteinaceous material.

Waxes

As observed, while the insect is extending its covering, the edge of the pygidium acts as a scraper to pick up cuticle wax from the plant surface. To obtain pure wax as secreted by the insect, a number of California red scale insects were grown on a thin celluloid film painted on grapefruit. These insects pierced the film with their stylets, but constructed their scale coverings entirely above the surface of the celluloid. Two thousand of these coverings, picked off and extracted, gave a small amount of pure scale-covering wax. This pure scale-covering wax melted at 86°–88° C., while the wax from the coverings of scale insects grown on the grapefruit surface melted at 83°–86°C. Cuticle wax from grapefruit melted at about 63° C. Apparently, the small amount of cuticle wax present in or on the coverings of the scales grown on grapefruits slightly lowered the melting point of the wax extracted from them. The melting point found for California red-scale wax is slightly higher than that reported for Chinese wax (80.5°–83.0° C.) from *Ericerus pe-la* Chavannes.

TABLE I

AVERAGE WEIGHT PER SCALE COVERING BEFORE AND AFTER FRACTIONATION

	Weight, micrograms
Original cover	18.1
Waxes extracted	7.6
Cover after benzene extraction	9.3
Handling loss during extraction	1.2
Secreted proteinaceous material (loss on treatment with NaOH)	7.9
Exuviae	1.4

Metcalf and Hockenyos (1930) reported melting points for waxes of the following armored scales: *Lepidosaphes ulmi* (L.), 94°–96°; *Chrysomphalus ficus* Ashm., 88°–90°; *Phenacaspis pinifoliae* (Fitch), 86°–88°. These melting points are all much higher than those reported for beeswax (62°–65°) or for most insect waxes that have been investigated. They are close to the 87° reported for myricyl cerotate ($C_{25}H_{51}CO.O.C_{30}H_{61}$), the chief constituent of carnauba wax, but also found in beeswax. Ceryl melissate ($C_{31}H_{63}CO.O.C_{27}H_{55}$), found in the wax of *Ceroplastes rubens* Maskell, melts at 86°.

A good compilation of information about insect waxes is given by Warth (1947). We may infer from published analyses of other scale waxes that the waxy portion of the covering of the red scale consists in great part of esters of long-chain, saturated, normal monohydric alcohols and corresponding or similar monobasic acids. The melting point indicates chains of 27 to 30 carbon atoms. The corresponding acids and alcohols are probably also present in small amounts, with possibly a trace of resins and hydrocarbons.

Proteinaceous Material

The proteinaceous part of the scale covering—that part not soluble in benzene—was tested in various solvents. At room temperature, sodium hypochlorite (1.3 percent) dissolved it rapidly, and concentrated NaOH dissolved it rather slowly. When kept at 45° C. for 24 hours, the scale coverings were dissolved by concentrated NaOH, 2 percent NaOH, concentrated HCl, and by 6 *N* HCl (partially). They were not dissolved by water, chloroform, 95 per cent ethyl alcohol, 70 percent ethyl alcohol, or 10 percent sodium chloride solution.

The proteinaceous material, or a part of it, is amphoteric, since a precipitate forms when an alkaline or basic solution is neutralized. It gave a positive xanthoproteic reaction, but was negative when Millon's reaction was tried. The test for cystine or cysteine sulfur was also negative, as was the Molisch test for carbohydrates.

SUMMARY

Aonidella aurantii (Mask.), an armored scale, forms its covering from exuviae and from material secreted by the glands in its pygidium. This material is secreted in the form of filaments and is formed into the shell-like covering by the action of its pygidium. During this period the insect rotates to produce the circular covering. This rotation is through not more than 540 degrees.

Scale coverings were found to consist, by weight, of about 8 percent exuviae, 45 percent waxes, and 47 percent secreted proteinaceous material. Pure scale-covering wax melted at 86°–88° C.

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This is a monograph of the South American mosquitoes of the genus *Haemagogus* with keys, descriptions, some ecological material, descriptions of laboratory and field techniques, and a bibliography. It is written in Spanish.

—M. T. J.

A BRIEF REVIEW OF THE PSYLLIIDAE OF PUERTO RICO

(Homoptera)

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Much more careful collecting and rearing needs to be done in this group before anything nearly complete can be presented for this area. The primary object of this paper is to lay a basis for the future by summarizing our limited knowledge of the psylliids of Puerto Rico and their relationship to the rest of the neotropical fauna. Other than accidental or incidental records there has been no effort to determine the fauna of the Caribbean region with the possible exception of Cuba and Puerto Rico. The recent records contained herein were acquired incidental to a survey by Caldwell and Martorell relative to bunchy-top disease of papaya, (*Carica papaya*). More nearly complete data from this survey for the auchenorhynchus Homoptera have been submitted for publication elsewhere.

We use the family name Psylliidae instead of Psyllidae because we have based our opinion upon the biological fact that Linnaeus did not have the psyllid of authors before him when he wrote his "Tenth Edition." Detailed discussions concerning the identity of *Chermes alni* Linnaeus have been given by Caldwell (1946) and Russell (1946).

Rather than repeat monotonous descriptions the known forms are identified by keys and a few pertinent remarks that may serve to separate one from another and are designed only for this region. Sequence in treatment does not necessarily indicate phylogeny. The family is badly in need of a thorough revaluation of generic concepts. There are eighteen forms, divisible into seven groups or genera, recorded from Puerto Rico. All genera are tropical or subtropical in nature and are strongly represented in Mexico and Central America with the possible exception of *Ceropsylla* which is poorly defined. To judge from the present records it would seem that the most successful ancestral forms were of pauropsylline stock. This includes *Euceropsylla* Boselli, which, while composed mostly of species formerly placed in the psylliines, nevertheless shows strong affinities to the pauropsyllines in both adults and immature stages. It is notable that no forms belonging in the Liviinae have been found; however, from the present records this group tends to be temperate or arctic in distribution.

KEY TO GENERA

1. Forewing with radius, media, and cubitus branching from basal vein at a common point, or nearly so. (Triozinae) ..... **Ceropsylla**
   Forewing with radius, media, and cubitus not branching from the basal vein at a common point 2
2. Head deeply cleft in front, median ocellus visible from above. (Carsidarinae) ..... **Carsidara**
   Head not deeply cleft in front 3
3. Vertex and head rounded forward; frons visible as a distinct sclerite; genae not conical. (Pauropsyllinae) 4
   Vertex and head not strongly rounded, more or less in one plane; frons concealed by genae which are more or less conical. (Psylliinae) 5
4. Forewing with broad, triangular pterostigma; forceps bifid apically ..... **Heteropsylla**
   Forewing with subcosta appearing as a distinct vein; forceps simple ..... **Paurocephala**
5. Vertex highly ridged along either side of median line ..... **Mitrapsylla**
   Vertex relatively smooth 6
6. Forewing clear, forceps bifid apically ..... **Euceropsylla**
   Forewing maculate; forceps simple ..... **Euphalerus**

Carsidara Walker

Carsidara concolor Crawford

1911. Pomona Jour. Ent. 3: 484.

A large elongate form easily recognized by the cleft head and the acute, apical angle of the forewing. It has been found along the south coast of Puerto Rico and on St. Thomas, V. I. The only other record is its topotypic locality, Cuba.

RECORDS: Salinas, at Río Jueyes, 11–21–47; Caja de Muertos Island, 12–11–47; St. Thomas, Virgin Islands, 11–29–47.

Ceropsylla Riley

KEY TO SPECIES

1. At least 3 mm. in length; greenish in color ..... **sideroxyli**
   Less than 3 mm. in length; reddish in color ..... **martorelli**

Ceropsylla sideroxyli Riley

1885. Proc. Biol. Soc. Wash. 2: 76.
1941. Jour. Dept. Agr. Puerto Rico 25: 54.
1949. Jour. Dept. Agr. Puerto Rico 29: 375.

The large size, greenish color, and the fact that it occurs on *Sideroxylon foetidissimum* separates this form from the other triozine in Puerto Rico. The cubitus separates from the main vein considerably before the radius and media.

RECORDS: The host tree of this species, "tortugo amarillo," "tortugo," or mastic, *Sideroxylon foetidissimum*, occurs in woodlands, river valleys and on hillsides at lower elevations in dry and moist districts of Puerto Rico. Wherever this tree occurs, *Ceropsylla sideroxyli* is present. The insect has been observed in Vieques, St. Thomas and Mona Islands.

Ceropsylla martorelli Caldwell

1942. Jour. Dept. Agr. Puerto Rico 26: 28–29.
1949. Jour. Dept. Agr. Puerto Rico 29: 375–376.

This is a small slender form that might be placed in *Trioza;* however the cubitus branches from the main stem before the radius and

media and the immature has sectasetae similar to those of *sideroxyli*. The small size and reddish color make recognition easy.

RECORDS: *Ceropsylla martorelli* attacks the leaves of the lauraceous trees, *Ocotea leucoxylon* and *Ocotea portoricensis*, locally known as "laurel geo" and "laurel," respectively. The insect occurs in low, middle and higher elevations following the habitat of its host trees.

Paurocephala Crawford

Paurocephala heterotrichi n. sp.

(Plate I)

Length, females approximately 1 mm., males a little smaller. General color gray. Forewing milky-hyaline, sometimes maculate with black, tending to be fumate apically; terminations of all veins dark.

Head broader than thorax. Eyes strongly recessive. Vertex with deep fovae. Genae very strongly swollen. Forewing little more than twice as long as broad; cubital cell very elongate, narrow; second branch of radius (or subcosta?) distinct, forming a marginal cell similar to cubital. Male proctiger hemipyriform. Forceps broad, obtuse apically. Female genital segment with dorsal valve little longer than ventral.

RECORDS: Male *holotype*, females *allotype* and *paratypes* from Rio Piedras, at low altitude, 8–25–47. *Paratypes* from Aguas Buenas, Enrique Castro's farm, altitude 1300 ft., 9–14–47; (Caldwell and Martorell). The species breeds on the tender shoots of "camasey de paloma" or "camasey colorado," *Heterotrichum cymosum*.

The very small size distinguishes this form from any other that is known to us.

Heteropsylla Crawford

The great majority of the heteropsyllids seem to be found in semi-arid habitats on members of the Mimosaceae. All forms are relatively small in size; the head is rounded anteriorly; and the genae though somewhat globose do not conceal the frons. Perhaps the most obvious character is the extremely broad pterostigma. With one known exception all males have the forceps bifid. The immatures seem to be active rather than quiescent and may often be observed running up and down the leaves or petioles.

KEY TO SPECIES

1. Forceps with outer arm one-fourth as long as inner ..... **reducta**
   Arms subequal in length, or with outer slightly longer 2
2. Forceps with outer arm inverted L-shaped ..... **puertoricensis**
   Outer arm more or less elliptical 3
3. Forceps with inner arm long, slender, evenly curved apically ..... **fusca**
   Inner arm stout, angulate apically 4
4. Forceps with outer arm almost truncate apically, posterior apical angle with a definite spur; inner arm inverted L-shaped ..... **cubana**
   Outer arm acuminate apically; inner arm inverted J-shaped ..... **huasachae** subsp. **insulata**

Heteropsylla reducta n. sp.

(Plate I)

Length, male 2 mm., forewing 1.7 mm.; female a fraction larger overall. Male brownish-testaceous to fuscous except the lateral angles

of the mesonotum; inner apical angle of forewings fumate to fuscous. Females greenish-yellow; forewings yellowish-hyaline.

Vertex with foveae scarcely impressed. Forewing twice as long as broad. Genital capsule of male very large. Proctiger as high as broad in lateral aspect. Outer arm of forceps much reduced, pyriform; inner arm four times as long as outer, apices acuminate, approximate. Female genital segment elongate.

This is the largest and most robust species in Puerto Rico. The vestigial condition of the outer arm of the forceps is unique.

RECORDS: Male *holotype*, females *allotype*, and *paratypes* from Guánica Insular Forest, 9–27–47, *paratypes* from Vieques Is., 10–22 and 10–23–47 (Caldwell & Martorell). The species breeds on the foliage of "mesquite," "algarrobo del Hawaii" or "bayahonda" tree, *Prosopis juliflora* (= *Neltuma juliflora*).

Heteropsylla puertoricensis Caldwell

1942. Jour. Dept. Agr. Puerto Rico 26: 28.
1949. Jour. Dept. Agr. Puerto Rico 29: 375.

This form is known only from the type series. The long stylate dorsal valve and short chunky ventral valve for the female and the inverted L-shaped outer arm of the forcep in the male are characteristic. The host tree is *Samanea saman*, locally known as "guango" or "saman," also called rain tree, giant thibet and cow bean tree in the Caribbean British Islands.

Heteropsylla fusca Crawford

1914. U. S. Nat. Mus. Bull. 85: 48.
1949. Jour. Dept. Agr. Puerto Rico 29: 345, 375 (det. as *mimosae*).

Both sexes dark fuscous to black; forewing yellowish. The cubital cell is slightly less arched than in *reducta*. The rather slender pyriform proctiger and slender, acuminate inner arms of the forceps are specific character. The female genital segment is elongate.

RECORDS: Collected near Salinas on the Cayey Road, breeding abundantly on the tender foliage of "campeche" or logwood tree, *Haematoxylon campechianum*, 11–15–47, at low altitudes.

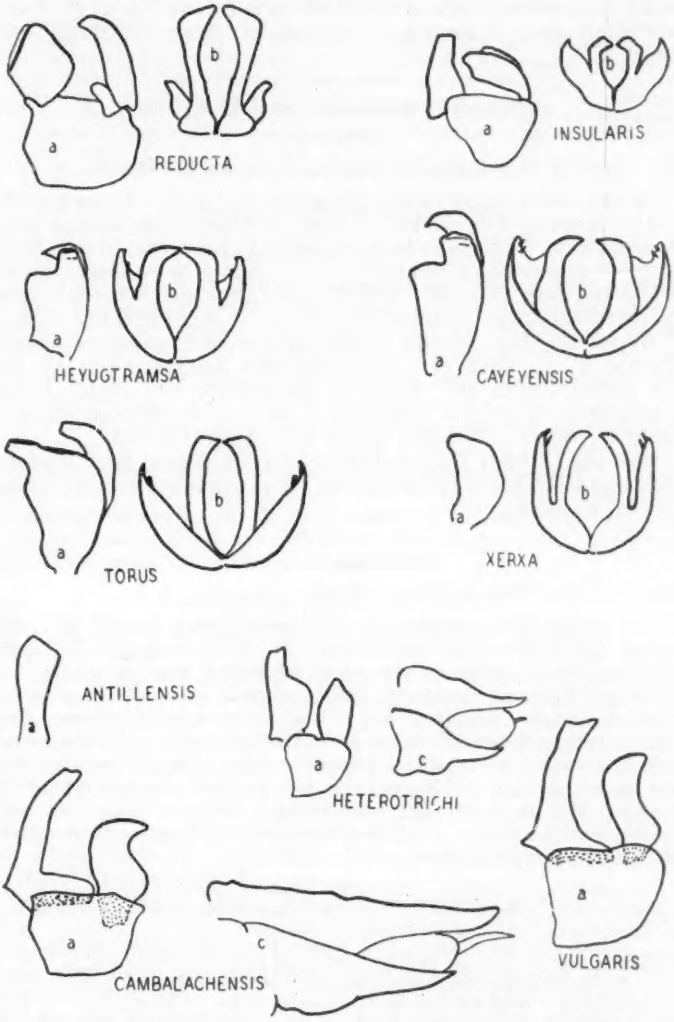
This form is also found in Haiti from sea level to 4500 ft. Adults were very abundant in January and were found on everything from clover to cactus. Topotypic locality: Dominican Republic.

Heteropsylla cubana Crawford

1914. U. S. Nat. Mus. Bull. 85: 46–47.
1945. Ent. News 61: 236–237.

An average-sized greenish-yellow form originally described from Cuba. The outer arm of the forceps has a definite apical spur posteriorly. The ventral valve in the female is proportionately shorter than in other forms excepting *puertoricensis*. Ponce, P. R., 8–28–47.

Crawford, in 1914, states, "Type in author's collection." Tuthill in 1945, evidently with the same material at hand, states that he is selecting one male as type since no type selection has been made. Through the courtesy of Sr. S. C. Bruner of Havana, Cuba, we have



1. Right lateral aspect of male genitalia or of forceps. b. Posterior aspect of forceps. c. Right lateral aspect of female genital segment.

received specimens determined as *cubana* by Tuthill so we have based our interpretation of *cubana* Crawford upon *cubana* Tuthill until such time examination of the original type should prove our interpretation invalid.

Heteropsylla huasachae insulata n. subsp.

(Plate I)

1941. Ohio Jour. Sci. **41**: 423 (Typical form, Mexico and Guatemala).

Length overall, male 1.8 mm., female 2 mm. General color greenish-fuscous; forewing milky-hyaline. Male proctiger rather slender, gently hemipyriform. Forceps as long as proctiger; outer arm ovate, slightly narrowed on anterior apical margin; inner arm of same height as outer with apices twice angulate. Female with dorsal valve lowered in apical half on dorsal margin; ventral valve three-fourths as long as dorsal.

RECORDS: Male *holotype*, female *allotype*, and *paratypes* from St. Thomas, V. I., 11–29–47. *Paratypes* from Aguirre, 11–21–47, from unidentified bushes and weeds at the Aguirre Golf Field; Vieques Island, 10–22–47 and Río Piedras, P. R., 8–18–47, breeding on the tender foliage of "acacia" or women tongue tree, *Albizzia lebbeck*.

The subspecies is a little smaller than the typical form, darker in color, and shorter in length of vertex. The genitalia are similar though different in detail.

Euceropsylla Boselli

1929. Lab. Zool. Gen. Agr. Portici **24**: 70.

The genus *Euceropsylla* with *russoi* as type was erected to contain *torrida*, *orizabensis*, and *minuticona*. It is characterized by species with the dorsal portion of the vertex depressed; the genae not overly developed; antennae usually as long as entire insect; forewings broadly rounded apically, usually short, and with the pterostigma very small. The forceps of the male are more or less bifid apically and the immatures have the anal opening apical. Host records indicate that this group occurs on members of Mimosaceae, usually *Inga*, throughout the neotropics. The genus is more compact and clear cut than the heterogeneous group known as *Psyllia* and certainly merits generic recognition. The known forms are listed:

bipartita (Tuthill, 1947). New combination (Costa Rica) (*Psylla*).
cayeyensis (Caldwell, 1942). New combination (Puerto Rico) (*Arytaina*).
heyugtramsa n. sp. (Puerto Rico).
martorelli (Caldwell, 1944). New combination (Puerto Rico) (*Psyllia*).
minuticona (Crawford). (El Salvador, Guatemala, and Mexico).
multiplex (Tuthill, 1947). New combination (Costa Rica) (*Psylla*).
orizabensis (Crawford). (Mexico).
russoi Boselli. (Santo Domingo).
torrida (Crawford). (Brazil).
torus n. sp. (Puerto Rico).
xerxa n. sp. (Puerto Rico).

It is possible that *Psylla itaporica* Crawford, (Brazil, 1925) belongs in this group.

KEY TO SPECIES

1. Female genital segment elongate, needle-like; male forceps with shallow apical notch ..... **martorelli**
   Female genital segment not needle-like; forceps deeply bifid apically 2
2. Outer arm of forceps narrowed from anterior margin subapically, step-like ... 3
   Outer arm of forceps more or less semilunate, not step-like 4
3. Inner arm of forceps longer than outer ..... **cayeyensis**
   Inner arm shorter than outer ..... **heyugtramsa**
4. Outer arm of forceps not produced anteriorly; inner and outer arms subequal in length ..... **xerxa**
   Outer arm produced anteriorly; inner arm longer than outer ..... **torus**

Euceropsylla martorelli (Caldwell)

1944. Jour. New York Ent. Soc. 52: 339.
1949. Jour. Dept. Agr. Puerto Rico 29: 377–378.

This form is the most highly modified (or possibly least modified) of all the euceropsyllids. The vertex is somewhat rolled forward and the genae are scarcely swollen and expose the frons which indicated close relationship to the pauropsyllines. The stylate female segment and the shallow forceps notch in the male sets this form apart from its close relatives.

RECORDS: Penón del Collao, on the Cayey-Salinas Road, 2000 ft. high, 8–28–47. Ponce-Adjuntas Road, Km. 24.6, altitude 1900 ft., 9–12–47; Jayuya-Ciales Road, Km. 23.2, altitude 1640 ft., 9–25–47; La Maravilla, Toro Negro Mts., 2800 ft., 11–14–47. The species breeds on the tender shoots of "guaba," *Inga vera*. This host tree is used extensively in Puerto Rico for coffee shade at low, middle and higher elevations.

Euceropsylla cayeyensis (Caldwell)

1942. Jour. Dept. Agr. Puerto Rico 26: 29–31 (including *minuticona*).
1949. Jour. Dept. Agr. Puerto Rico 29: 376–377 (including *minuticona*).

Cayeyensis seems to be the most abundant form in Puerto Rico and since it occurs on the same host, *Inga vera*, as *martorelli*, *heyugtramsa*, and *xerxa*, we can only assume that the most abundant immature is also this form; therefore, references to *minuticona* from Puerto Rico must be referred to *cayeyensis*. The only reliable criteria for recognition is the step-like outer forceps arm which is shorter than the inner arm. Separation of the female of the above listed forms is possible by observance of the profile of dorsal female valve but there is some question as to which female belongs to which male and again we have had to base our decision on frequency and locality.

RECORDS: Carite Mts., at higher elevations, 10–2–47. Jayuya-Ciales Road, Km. 23.2, altitude 1740 ft., from *Inga vera*, 9–25–47; Ciales-Jayuya Road, Km. 30.6, 1820 ft. altitude from *Guarea*, *Dendropanax* and *Inga vera*, 9–25–47; Ponce-Adjuntas Road, Km. 23.7, altitude 2000 ft. from *Inga vera*, 9–12–47; Penón del Collao, Cayey, 2000 ft. high, 8–28–47; from *Inga vera*. Orocovis, at river bed near town, 1900 ft. high, swept from weeds and grasses, 9–11–47; Barranquitas-Aibonito Road, Bo. Helechal, Km. 8.4, 1900 ft. high, from *Solanum Rubus*, *Ipomoea* and *Casearia*, 9–11–49; Ponce-Adjuntas Road, Km. 24.6, altitude 1900 ft., from *Inga vera*, 9–12–47; Jayuya in front of sugar mill, swept from "alelaila," *Melia azedarach*, 9–25–49.

Euceropsylla heyugtramsa n. sp.
(Plate I)

Length overall, male 2.5 mm., female 2.8 mm. General color greenish-yellow. Chrotic characters not sufficiently different than those of related forms for recognition. Forceps relatively short, broad; outer arm broadly step-like apically; outer and inner arms of equal length, united at midlength posteriorly. Dorsal valve of female shorter, straighter than in *cayeyensis*.

RECORDS: Male *holotype*, female *allotype* and *paratypes* from Adjuntas, P. R., at middle elevations, swept from unidentified bushes, 9–12–47; (Caldwell and Martorell); *paratypes* Maricao, 7–2–17 (Morrison) and Bayamon, P. R., (Anderson and Lesesne).

The word "heyugtramsa" is an expression of the natives in the region where the species was taken, and according to I. W. Harper, is freely translated "wise one."

Euceropsylla xerxa n. sp.
(Plate I)

Length overall, male 2.3 mm., female 2.5 mm. General color greenish-yellow with five broad but faintly indicated orangish stripes on mesonotum; forewing yellowish.

Vertex less than twice as broad as median length. Genae conical, over half as long as vertex. Forewing twice as long as broad; pterostigma rather broad at base. Male proctiger gently pyriform. Forceps semilunate in lateral aspect; inner arm not visible laterally but is slender and acuminate apically. Valve of female with dorsal little longer than ventral.

RECORDS: Male *holotype*, female *allotype*, and *paratypes* from Vieques, P. R., 10–22–47; paratypes from Guánica, P. R., 1–6–48; (Caldwell and Martorell).

This is the smallest species of euceropsyllid in Puerto Rico and the only one found in a xerophytic habitat.

Euceropsylla torus n. sp.
(Plate I)

Length overall, male 2.5 mm., female 3 mm. General color greenish-yellow with indications of stripes on the mesonotum.

Vertex twice as broad as median length. Genae elongate-globose, less than half as long as vertex. Forewing about twice as long as broad; pterostigma very small; cubital cell highly arched. Proctiger of male greatly narrowed in apical fourth posteriorly. Outer arm of forceps broadly truncate above; apex narrowed and projected anteriorly; inner arm longer than outer. Female genital segment similar to *cayeyensis* but longer.

RECORDS: Male *holotype*, female *allotype* and *paratypes* from La Maravilla, Toro Negro Mts., P. R., 2800 ft., from unidentified plants, 11–14–47; (Caldwell and Martorell).

Mitrapsylla Crawford

The species in this group are differentiated from the general group of psylliines by the peculiar ridges that parallel the medial suture

on the vertex. The antenna are usually elongate and the eyes usually recessive. In the males known to us the proctiger supports some sort of a caudal flap on either posterior margin. Our limited host knowledge suggests that most forms occur on members of the Mimosaceae.

The known forms are listed:

albalineata Crawf. (El Salvador, Mexico, and Nicaragua).
cedusa (Caldwell, 1944). Mexico). New combination (*Psyllia*).
cubana Crawf. (Cuba).
deserata Cald. (Mexico).
cambalachensis n. sp. (Puerto Rico).
vulgaris n. sp. (Puerto Rico).
unga (Caldwell, 1942). (Puerto Rico). New combination (*Arytaina*).

KEY TO SPECIES

1. Female with dorsal valve much longer than ventral, bearing a deep notch in dorsal surfaces ..... **unga**
   Female with dorsum of dorsal valve straight and without notch 2
2. Female with dorsal valve much longer than ventral. Male with forcep strongly S-shaped posteriorly ..... **cambalachensis**
   Female with valves subequal in length. Male with posterior margin of forcep gently undulant ..... **vulgaris**

Mitrapsylla vulgaris n. sp.

(Plate I)

Length overall, male 2.2 mm.; female 2.5 mm. General color brownish-yellow; with ridges of vertex, six stripes across pronotum, median stripe across mesonotum, and five stripes on praescutum whitish. Forewing clear with black marginal spots at termination of clavus, between branches of cubitus, between cubitus and media, and between branches of media.

Vertex twice as broad as median length. Antennae three times as long as width of vertex. Forewing little more than twice as long as broad; pterostigma very short. Proctiger of male with elongate basal flap on either posterior margin. Forceps elongate, gently undulate posteriorly. Female valves subequal in length.

The robust genital segment of the female with both valves subequal in length separate *vulgaris* from any known form.

RECORDS: Male *holotype*, female *allotype*, and *paratypes* from Adjuntas, P. R., on the Ponce-Adjuntas Road, Km. 27.9, altitude 1550 ft., 9–12–47; *paratypes* from Rio Piedras, in front of Central San José along railroad line, 8–17–47; and Ponce, P. R., 10–9–47. Also observed at Juana Díaz near Losey Field, Km. 3.7, Juana Díaz-Losey Field Road, 10–9–47; La Maravilla, Toro Negro Mts., 3700 ft. high, 11–14–47; Colonia Juana Díaz almost at sea level near Central Cortada at Santa Isabel, 5–20–50. The insect breeds on the young and tender foliage of "moca," bastard mahogany or bastard cabbage tree, *Andira jamaicensis* (=*Andira inermis*), from sea level to higher altitudes up to 4000 ft.

Mitrapsylla cambalachensis n. sp.

(Plate I)

Length over all, male 2.5 mm.; female 2.8 mm. Color and marking similar to *vulgaris*. Male proctiger slender; basal flap broader than

proctiger. Forceps strongly S-shaped posteriorly; apex appearing truncate with true apex projecting posteriorly in lateral aspect. Dorsum of dorsal valve in female straight, elongate, level in lateral aspect. Ventral valve slightly bulbose basally, much shorter than dorsal valve.

RECORDS: Male *holotype* and female *allotype* from Cambalache Experimental Forest, P. R., 100–250 ft., 11–6–47; (Caldwell and Martorell).

Mitrapsylla unga (Caldwell)

1942. Jour. Dept. Agr. Puerto Rico 26: 30–31.

Male unknown. The elongate genital segment of the female is similar to that of *cambalachensis* except there is a decided depression caudad to the anal opening. The anal opening is proportionally smaller and much less elliptical. The forewing more nearly resembles that of *vulgaris*.

RECORDS: Other than the record of the type, we have one specimen from Aguas Buenas, P. R., Mr. Enrique Castro's farm, altitude 1300 ft., from unidentified melastomaceous shrubs, 9–14–47.

Euphalerus Schwarz

Euphalerus antillensis n. sp.

1904. Proc. Ent. Soc. Wash. 6: 153–154 (Cuba record of *nidifix*).
1914. U. S. Nat. Mus. Bull. 85: 119–120 (Cuba record of *nidifix*).
1943. Iowa Jour. Sci. 29: 520 (Cuba and Virgin Is. record of *nidifix*).
1949. Jour. Dept. Agr. Puerto Rico 29: 376 (Puerto Rico record of *nidifix*).

Length, male 2 mm., female 2.5 mm.; forewing, male 1.5 mm., female 1.8 mm. General color yellowish, maculate overall with small black spots. Forewings more densely maculate and fumate apically.

Vertex twice as broad as long. Genae as long as vertex at median line, bluntly rounded. Head, pronotum, and praescutum with less slope than in *nidifix*. Forewing twice as long as broad. Proctiger of male broadly lobate on either lateroposterior margin. Forceps slightly diagonally truncate apically; apices approximate. Dorsal valve of female narrowed in apical fourth; ventral valve little shorter than dorsal.

This species differs from any other Puerto Rico psylliid by the dense maculations over the entire body and forewings. It differs from *nidifix* by the lesser slope of head and thorax, denser maculae on forewings, less stylate dorsal valve in the female, and the truncate forceps in the male. Since the type of *nidifix* is supposedly lost and the original series contained material from both Florida and Cuba we have to assume that Schwarz did his rearing work in Florida and that the type was also from there. Crawford noted the differences between Cuban and Floridan material and it is probable that the Cuban forms are specific with the Puerto Rican.

RECORDS: Male *holotype*, female *allotype*, and *paratypes* from Guánica, on the Guánica-Guánica Central Road, in front of stone quarry, breeding abundantly on the undersides of the leaves of "geno-geno," *Lonchocarpus domingensis*, 8–28–47; (Caldwell and Martorell).

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NATURE'S WAYS, HOW NATURE TAKES CARE OF ITS OWN, by Roy Chapman Andrews. 205 pages, 72 halftones and 72 full color illustrations. Crown Publishing Company, New York, 1951. Price, $3.75.

This unusual book relates many facts about curious animals, odd birds, bizarre fish and interesting insects, which it introduces in an appealing and stimulating fashion. It presents, particularly the self protective devices of Nature's children along with other interesting facts about their lives and habits. Thus *Nature's Ways* will fill a great need for a comprehensive, graphic and simplified book to present to children, young and old, these little known facts about creatures all around us. Teachers will find it a very good book to use in helping to arouse an interest in the out of doors.

Dr. Andrews, himself, is a sincere and ardent lover of nature, who has traveled many paths of the world to satisfy his interest and curiosity. His many keen observations and unusual interpretations of animals at home are shared most interestingly in his book. Andre Durenceau, his illustrator, with exceptional pictures has made *Nature's Ways* an artistic, as well as verbal, interpretation. It appeals to quite small children, as well as to older persons, as we have found by experience.

There are 26 plates dealing with entomological subjects alone. The rest cover animals of different phyla.—Helen B. James.

REVIEW OF THE WING VENATION OF THE HIGHER HYMENOPTERA (SUBORDER CLISTOGASTRA), AND SPECULATIONS ON THE PHYLOGENY OF THE HYMENOPTERA

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I. STRUCTURAL CHARACTERISTICS OF THE SUBORDERS CHALASTOGASTRA AND CLISTOGASTRA AND THEIR EVOLUTIONARY SIGNIFICANCE

Very early in the study of the systematics of Hymenoptera, a division of the order into two groups was established—a smaller and more primitive group in which the larval stages are plant feeders, and a much larger group with larvae of diverse feeding habits (primarily zoophagous, but some secondarily phytophagous). This classification is still used, and the suborders are, respectively, the Chalastogastra (also called Symphyta, Phytophaga, or Sessiliventres) and Clistogastra (also designated Apocrita or Petiolata). Some structural features of these two suborders are summarized as follows:

CHALASTOGASTRA.—Larvae of the externally feeding forms caterpillar-like, with thoracic legs and abdominal prolegs, but some living in protected situations lack the prolegs or may even be entirely apodous; mandibles well developed, with the associated musculature necessitating a well-sclerotized head capsule; ocelli frequently present; digestive tract continuous. Adult with abdomen not constricted near the base; trochanters always apparently two-segmented; venation of hind wing relatively complex, *i.e.*, with three basal cells, resulting from the persistence of the second vannal vein and the vannal cross-vein; posterior margin of hind wing not deeply incised to form strong lobes.

CLISTOGASTRA.—Larvae apodous, although a few forms have fleshy ventral projections on the thoracic or abdominal segments, or both; mandibles usually weak, and head capsule rarely heavily sclerotized; digestive tract not continuous, the mid-intestine not connected with the hind gut. Adult with abdomen more or less constricted near the base, often strongly so, with the first segment completely integrated with the thorax, and the second (apparently the first) abdominal segment narrowed at least anteriorly. Trochanter apparently two-segmented in the more primitive groups, but one-segmented in the rest. Hind wings with venation simpler, *i.e.*, with fewer than three basal cells, as a result of the loss of the second vannal vein (except in some barconids) and the vannal cross-vein; in the terebrantian groups, the posterior margin of the hind wings is usually entire, but in the more advanced groups there are typically one or two deep incisions which produce one or two lobes.

Larvae of the leaf-feeding Chalastogastra superficially have a strong resemblance to the larvae of Lepidoptera, which also are mostly leaf feeders. Perhaps the most significant difference in the mode of life of these two groups is the fact that in the phytophagous Hymenoptera the soft-shelled unornamented eggs are placed within the plant tissue by means of a specially modified ovipositor. In the Lepidoptera, the eggs are usually laid externally on the host plant, are usually heavily shelled and often strengthened by roughenings and ridges (usually termed "ornamentation"), and in only a very few forms is the ovi-

positor modified so as to function in piercing plant tissue. The piercing hymenopterous ovipositor, originally adapted for penetrating plant tissue, is a structure of fundamental importance for the whole order, proving to be a suitable instrument for inserting eggs in the arthropod hosts of the parasitic forms, for a weapon of offense and defense in the predaceous forms, and for a defensive weapon in the pollen-collecting bees.

It seems probable that the suborder Clistogastra evolved from some wood-boring chalastogastran by way of a parasitic group, with an intermediate stage in this evolutionary development being represented, in a general way, by the Oryssidae, which are placed in the Chalastogastra on the basis of structure, but have larvae that are internal parasites of wood-boring beetles. This concept of the origin of the Clistogastra is that outlined by Brues (1921). The parasitic larvae lose the caterpillar-like structures of the chalastogastran larva which fit it for a leaf-feeding mode of life. One might expect that the adoption of a parasitic mode of life by a group of animals would limit the future evolutionary possibilities of the group to exploitation of parasitism, since there is usually degeneration of the structures necessary only for a free-living existence. However, in the parasitic Hymenoptera, these "degenerative" adaptations have affected mostly the larvae, and, further, have not made it impossible for the larvae to live under non-parasitic but especially favorable conditions established by parental care. In the Hymenoptera, the evolution of a parasitic group from primitive plant-feeding forms laid the basis for the development of a large and diverse group which contains various parasitic forms, gall makers, predaceous wasps, and pollen-collecting bees.

The most characteristic structural feature of adults of the suborder Clistogastra is the constriction near the base of the abdomen, the "wasp waist," in which the first segment becomes fused with the thorax, and either the anterior end of or the entire second segment becomes very narrow. The functional significance of this structure may be connected with the need for relatively great arc, strength, and preciseness in downward movement in stinging the more or less active host or prey. This narrowing could presumably not have taken place between the first abdominal segment and the thorax because no muscles originating in the thorax could be allocated to the function of abdominal movement, since they had already been committed to the operation of the legs and wings.

Although wing venation furnishes important characters in the classification of Hymenoptera at the level of families and superfamilies, it cannot be made the sole basis of classification, since different modes of flight have not been the primary factor in determining different modes of life within the order. For example, the Sphecoidea do not differ from the Apoidea with respect to wing venation, but do differ in that the Apoidea have pollen-collecting structures, although the males and some secondarily parasitic bees lack them, as well as some bees that transport pollen in the digestive tract. It is said that all bees, even if lacking pollen-collecting structures, have at least a few branched hairs on the body, and that none of the wasps have these.

There is, however, a structure concerned with the wings found in the bees, and not in wasps, which is described in detail by Snodgrass (1942: 54, 63). This is an endoskeletal structure termed the axillary lever, which is present in bees and which facilitates the rapid flexing of the front wings over the abdomen after the insect alights. The advantages of folding the wings back after alighting are obvious, and bees must make very frequent landings and take-offs while collecting pollen and nectar.

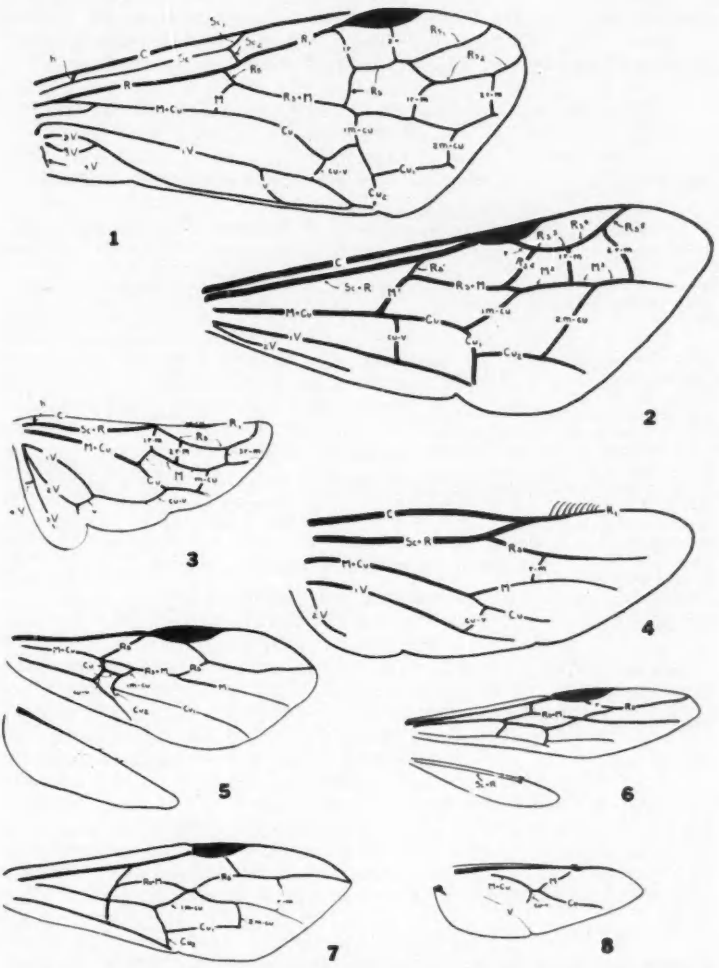
The adaptive value of certain minor characteristics of wing venation at the level of families and genera is at least indirectly illustrated by the stability of venation within groups which may contain many hundreds of species. In the Apoidea, for example, many genera can be distinguished by extremely minor venational characteristics, like the square second submarginal cell of *Nomia*, the arched basal nervure of *Halictus*, or the slightly sigmoid second recurrent nervure of *Colletes*. At the same time, other parts of the venation may show considerable individual variation.

The aerodynamics of insect flight in relation to differences in musculature, articulation, wing shape, and venation offers a large field of investigation. The consequences of different patterns of venation on the physical characteristics of the wing membranes need to be investigated by direct experimental methods. Stresses acting upon the insect wing during flight would come not only from air resistance but also from rapid changes in direction of the motion of the wings. These together would be expected to distort the wing membrane, with the amount and kind of distortion dependent in large part upon the venation. Just as the elasticity of the thoracic exoskeleton plays an important part in the application of motive force to the wing, the elasticity of the wing membrane, as modified by the venation, might play an important part in determining the aerodynamic characteristics of the wing.

The Hymenoptera showing the most rapid and precisely controlled flight are the bees and wasps. The venation of these is not highly reduced, but retains nearly all of the veins, in the front wing at least, that would appear in a hypothetical clistogastran ancestor. However, with some exceptions, the Sphecoidea and Apoidea have the trailing edge of the flight surface (in the Hymenoptera the front and hind wing on each side are held together during flight by a set of hooks, so that both function as a single plane) incised with one or two deep notches, which produce one or two strong lobes. It is noteworthy that the same development has taken place in the Diptera, where the more primitive, slow-flying groups have the hind margin of the wing entire, and the more advanced, powerfully flying groups have the trailing edge incised.

II. TERMINOLOGY OF WING VEINS OF THE HYMENOPTERA

The terminology of the wing veins of Hymenoptera proposed by Ross (1936) has been applied by Burks (1938) to the Chalcidoidea, Duncan (1939) to the Vespidae, Michener (1944) to the Apoidea, and Riegel (1948) to the Braconidae, the latter worker treating a varied group in considerable detail. This terminology is used here, with certain modifications introduced by Duncan and Michener. No



1. Generalized hymenopterous front wing (after Ross, slightly modified). 2. Generalized clistogastran front wing. 3. Xyelid hind wing (composite of *Xyela* and *Macroxyela*). 4. Generalized clistogastran hind wing. 5. *Gasteruption* (Gasteruptionidae); front and hind wings. 6. *Stephanus* (Stephanidae); front and hind wings. 7. *Oleisopristor* (Aulacidae); front wing. 8. *Oleisopristor* (Aulcidae); hind wing.

attempt is made to treat in any detail the varied modifications in venation resulting from different modes of reduction within certain families, notably the Braconidae and Formicidae; rather, only the more generalized members of the superfamilies or families are dealt with.

TERMINOLOGY OF THE WING VEINS IN A GENERALIZED CLISTOGASTRAN WING

(Figs. 2, 4)

Name of Vein, Based on Ross's Revision of Comstock-Needham System	Symbol	Descriptive Name
Costa	C	Costal
Subcosta + Radius	Sc + R	Subcostal
First abcissa of Radial sector	Rs^1	Parastigmal (cubitus of Jurinean system should not be used)
First abscissa of Media	M^1	Basal
Second abscissa of Radial sector	Rs^2	First intercubital
Radial cross vein	r	Marginal or radial
Third abscissa of Radial sector	Rs^3	
Fourth abscissa of Radial sector	Rs^4	
Fifth abscissa of Radial sector	Rs^5	
First radio-medial cross vein	1 r-m	Second intercubital
Second radio-medial cross vein	2 r-m	Third intercubital
Media + Cubitus	M + Cu	Median
Radial sector + Media	Rs + M	Submarginal (cubital of Jurinean system should not be used)
Second abscissa of Media	M^2	
Third abscissa of Media	M^3	
Fourth abscissa of Media	M^4	
First abscissa of Cubitus	Cu^1	Discoidal
Second abscissa of Cubitus	Cu^2	
Third abscissa of Cubitus	Cu^3	
Second branch of Cubitus	Cu_2	
First branch of Cubitus	Cu_1	Subdiscoidal
First medio-cubital cross vein	1 m-cu	First recurrent
Second medio-cubital cross vein	2 m-cu	Second recurrent
Cubito-vannal cross vein	cu-v	Nervulus or transverse medial
Vannal	V	Vannal or anal
Hind wing		
Costa	C	Costal
Subcosta + Radius	Sc + R	Subcostal
Radial sector	Rs	Marginal or radial
Radio-medial cross vein	r-m	Transverse cubital
Media + Cubitus	M + Cu	Medial
Media	M	Submarginal (cubital should not be used)
Cubitus	Cu	Discoidal
Cubito-vannal cross vein	cu-v	Transverse medial
Vannal	V	Vannal or anal

A classical descriptive terminology for the wing veins of Hymenoptera has long been used in the literature, a terminology largely based on that established by Jurine in the first quarter of the nineteenth century. This terminology has proved very useful, including a general termi-

nology applicable to many groups and specialized terminologies for wings with reduced venation, like those of the Braconidae, Ichneumonidae, and Chalcidoidea.

An extensive glossary of the terms applied to both the veins and cells of the hymenopterous wing, totalling about 600 entries, has been compiled by Rowher and Gahan (1916).

Equivalents in the descriptive terminology for Ross's morphological terms are given in the following table, which lists the veins of the generalized clistogastran wing; this generalized type is found little or not at all modified in most of the Scolioidea, Pompiloidea, Vespoidea, Sphecoidea, and Apoidea. Abscissas of the veins are numbered by means of subscripts.

Names of the cells may be found in the explanation for figure 27.

Some minor innovations in the descriptive terminology are introduced in the table. The terms "cubitus" and "cubital" should not be used in the Jurinean sense, since they conflict with Cubitus of the Comstock-Needham system. Cresson's term "parastigmal" is substituted for "cubitus," and "submarginal" is used for "cubital," the vein extending under the submarginal cells. Michener (1944: 181) points out that the term "cubital cells" should not be used for the submarginal cells, since Ross applies the term to cells associated with Cubitus; however, he retains the name "intercubitals" for the veins between the submarginals, and the term is used here, for want of a more appropriate name.

III. REVIEW OF WING VENATION OF THE CLISTOGASTRA

A front wing with the venation necessary to serve as a hypothetical ancestral type for the Clistogastra is shown in figure 2. A comparison of this wing with that of the hypothetical generalized hymenopteran wing proposed by Ross (fig. 1) shows that little modification is involved in the derivation of the basic clistogastrous type. In the front wing, Sc disappears, presumably by fusion with R. Either 1 or 2 r is lost and a branch of Rs is lost. Basally, Cu completely fuses with M. Of the venation posterior to the first vannal, only the second vannal remains, and then occurs only rarely (*i.e.*, in some braconids). Ross does not figure an ancestral hymenopterous hind wing, but that of a xyelid (fig. 3, actually a composite of *Xyela* and *Macroxyela*) will serve as a representative. Compared with this wing, the ancestral clistogastrous hind wing has lost 2 and 3 r-m, m-cu, and all venation posterior to 2V (the second vannal occurs rarely, as in some braconids). Basally, Cu is completely fused with M.

The classification of Hymenoptera used here is that of Brues and Melander (1932), and is summarized as follows, down to the level of superfamilies:

Order Hymenoptera
- Suborder Chalastogastra
  - (Pamphilioidea, Tenthredinoidea, Siricoidea, Oryssoidea)
- Suborder Clistogastra
  - Division Terebrantia
    - (Ichneumonoidea, Chalcidoidea, Proctotrupoidea, Cynipoidea)
  - Division Aculeata
    - (Bethyloidea, Chrysidoidea, Scolioidea, Formicoidea, Vespoidea, Pompiloidea, Sphecoidea, Apoidea)

Division **Terebrantia**

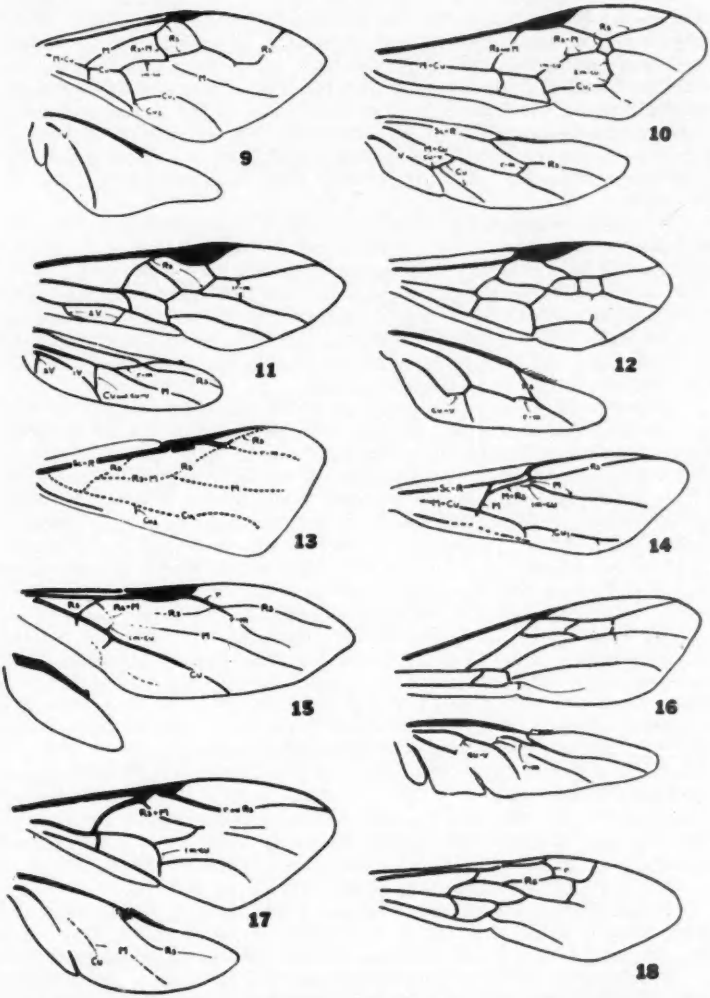
As was previously stated, the primitive suborder Chalastogastra is generally considered to have given rise to the higher Hymenoptera by way of parasitic forms similar to the Oryssidae. The suborder Clistogastra, then, would be in its inception a parasitic group, with the Ichneumonoidea representing the most primitive superfamily. The rest of the Terebrantia—the chalcidoids, proctotrupoids, and cynipoids, can probably be derived from the ichneumonoids. Most of the ichneumonoids (the braconids and ichneumonids) are too specialized in regard to wing venation as well as other structural features to be considered as representative of the ancestral aculeates, but some more generalized families, especially the Trigonalidae and Aulacidae, are structurally much like a hypothetical ancestral aculeate, especially with respect to the venation of the front wings. Some generalized braconids have the very primitive feature of retaining a second vannal in both the front and hind wings, but the rest of the venation is moderately reduced. Venation of hind wings of living ichneumonoids is much more reduced than that of the basic clistogastrous type. Probably most of the ichneumonoids have the vannal area of the hind wing more reduced than that of the ancestral clistogastran.

Superfamily **Ichneumonoidea**

Megalyridae.—Although this family is considered to be a primitive one, the venation is rather highly reduced. In *Dinaspis*, as figured by Waterston (1922), a long basal portion of M is missing, 2 r-m is missing, with 1 r-m indicated only faintly, the apical portion of Cu is missing, and both 1 and 2 m-cu are missing in the front wing. The hind wing is even more specialized, with only a single longitudinal vein, probably Sc + R, remaining. A primitive feature of the front wing is the wide separation of C and Sc + R, leaving a costal cell.

Stephanidae.—The venation of this otherwise primitive family shows moderately strong reduction. *Stephanus* (fig. 6) retains only one intercubital vein, here more or less arbitrarily called Rs, and has lost 2 m-cu. The slender hind wing lacks venation, except for (Sc + R), which closely parallels the margin of the wing. Only three hamuli are present.

Aulacidae.—Of the ichneumonoid families considered thus far, the venation of this is most generalized (fig. 7). In comparison with the ancestral clistogastrous type, the front wing has lost only an intercubital vein. The veins in the region behind the stigma in all the Hymenoptera are difficult to interpret, as Ross has pointed out, and assignment of homologies to them is more or less arbitrary. Reigel (1948: 444) gives evidence for regarding the vein bounding the outer margin of the first submarginal cell in a very similar braconid wing as a serial vein composed of 2 r-m and the distal portion of the second abscissa of Rs. The approximation of the upper end of Rs^2 and 2 r-m in some trigonalid wings also indicates the possibility that there is some kind of fusion of these veins in the ichneumonoids with a reduced number of intercubitals. However, in the interests of a simplified terminology, the vein bounding the external margin of the first submarginal cell is termed Rs.



9. *Evania* (Evaniidae); front and hind wings. 10. *Pterocormus* (Ichneumonidae); front and hind wings. 11. *Gymnoscelis* (Braconidae); front and hind wings. 12. *Lycogaster* (Trigonalidae); front and hind wings. 13. *Spilochalcis* (Chalcididae) (after Burks); front wing. 14. *Ibalia* (Ibaliidae); front wing. 15. *Pelecinus* (Pelecinidae); front and hind wings. 16. *Rhopalosoma* (Rhopalosomatidae); front and hind wings. 17. *Chrysis* (Chrysididae); front and hind wings. 18. *Scolia* (Scoliidae); front wing of male.

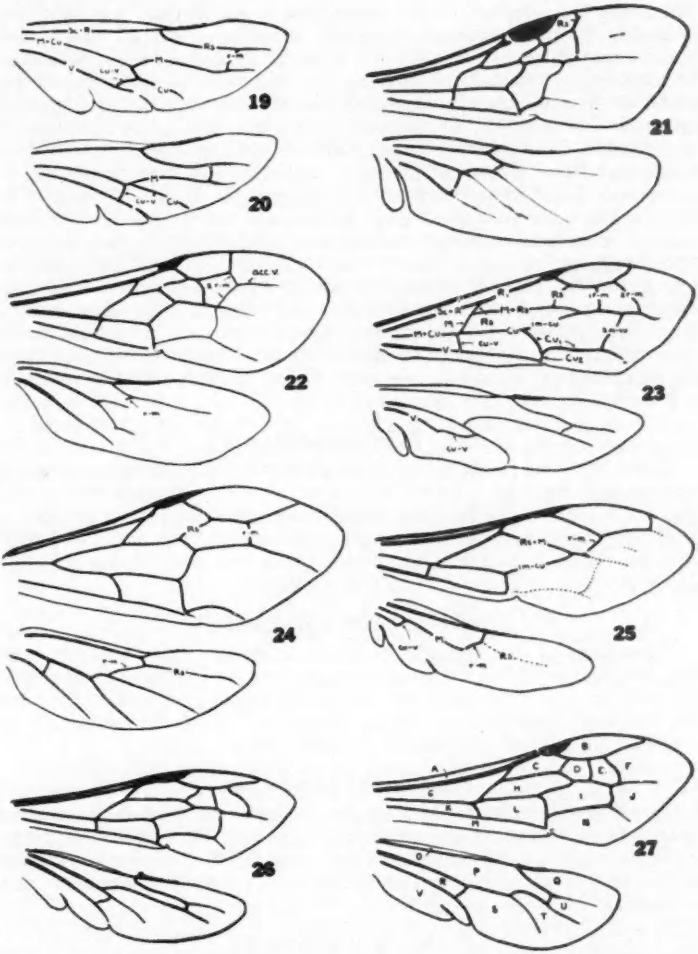
Gasteruptionidae.—A peculiar appearance is presented by the front wing of *Gasteruption* due to the presence of two cells beyond what appears to be the cubito-vannal cross-vein. This can be accounted for if it is assumed that Rs + M has been pulled back until it is in line with the trunk of M + Cu, and that the first abscissa of the apparent cu-v is actually the first abscissa of Cu_1. This is the interpretation of Rohwer and Gahan (192: fig. 9), although they use a different terminology. There seems to be no good reason for not calling the single intercubital vein Rs. The front wings fold longitudinally, as in the Vespidae.

Evaniidae.—As in the preceding families, C is separate from Sc + R, enclosing a narrow costal cell. The most primitive venation known to me to occur in this family is somewhat reduced, with 2 r-m and 2 m-cu missing. The wing in figure 9 has the venation more reduced. The hind wing is remarkable among the ichneumonoids in possessing a well-formed jugal lobe. In this respect the family leads to the structure of the aculeates, and may well be rather closely related to the ancestral stock of the aculeates and terebrantians.

Ichneumonidae.—As illustrated in figure 10, the venation of this extensive family is rather strongly reduced. The venation is quite stable within the family, variation in the front wing mainly involving the loss of one of the two remaining intercubitals. Reduction to the ichneumonid type has probably taken place by the loss of the basal portion of Rs + M; a remnant is sometimes seen as a spur directed medially from the end of 1 m-cu. The first vein extending from Cubitus to the submarginal cell ("areolet") is then actually a serial vein composed of 1 m-cu and the distal portion of Rs + M. The descriptive term "cubito-discoidal" is sometimes applied to this vein.

Braconidae.—In contrast to the stability of venation in the Ichneumonidae, that of the Braconidae shows great diversity, with extreme reduction taking place. Reigel (1948) has traced out the mode of reduction in detail in numerous genera; only the more primitive braconid venation is considered here. The front wing of *Gymnoscelis* (fig. 11) can be derived from the trigonalid type by the loss of an intercubital and the second recurrent nervure. A primitive and unique (among the Clistogastra) feature of the venation of this and related braconids is the retention of some venation posterior to the Vannal. Scarcely any venation remains in the small aphidiines. A specialized feature of both braconid and ichneumonid front wings is the fusion of C and Sc + R, obliterating the costal cell.

Trigonalidae.—Inspection of figure 12 will show that the trigonalid front wing is much like that of the clistogastrous ancestral type; only the braconid Vannals are lacking. It has long been recognized that the family Trigonalidae combines ichneumonid with aculeate characters, and this may indicate, not that it should be regarded as the most highly evolved ichneumonoid, but that it is much like the primitive stock from which both the Aculeata and Terebrantia evolved. However, the highly aberrant biology of the trigonalids seems to contradict the generalized structure of these insects. In most trigonalids, the egg is first swallowed by a caterpillar or sawfly larva. After hatching in the digestive tract of this host, the trigonalid larva seeks out a dipterous or



19. *Myzine* (Tiphiidae); hind wing of male. 20. *Myzine* (Tiphiidae); hind wing of female. 21. *Brachycystis* (Mutillidae); front and hind wings. 22. *Dasymutilla* (Mutillidae); front and hind wings. 23. *Batazonus* (Pompilidae); front and hind wings. 24. *Eciton* (Formicidae); front and hind wings. 25. *Solenius* (Crabronidae); front and hind wings. 26. *Philanthus* (Philanthidae); front and hind wings. 27. *Colletes* (Colletidae).—A, costal cell; B, marginal or radial; C, first submarginal; D, second submarginal; E, third submarginal; F, fourth submarginal; G, medial; H, first discoidal; I, third discoidal; J, second apical; K, submedial; L, second discoidal; M, vannal; N, first apical; O, costal; P, median; Q, marginal or radial; R, submedian; S, subdiscoidal; T, discoidal; U, submarginal; V, vannal.

hymenopterous parasite in the caterpillar or sawfly larva, in which it completes its development. In others, parasitic on vespid wasps, it is thought that the eggs are fed to the wasp larva in the process of feeding the larva with infected caterpillars; the trigonalid parasite then completes its development in the wasp larva. An Australian trigonalid shows the most primitive life history in the family, completing its development in a sawfly larva, without seeking out a second host (Clausen, 1940). The most plausible origin for this type of parasitism seems to be that it was secondarily derived from the typical ichneumonoid type, in which the parasite egg is laid directly on or in the host. There does not seem to be any easy transitional stage whereby a phytophagous form could evolve into a parasite of the trigonalid type, whereas the scheme for the origin of parasitism outline by Brues does offer an easy transition from a wood-eating form to one feeding on other wood-borers. If the trigonalids could be shown to represent an early stage in the evolution of parasitism in the Hymenoptera, there would have to be drastic reorganization of the phylogeny of the group, probably resulting in a diphyletic origin for the Terebrantia.

Superfamily **Chalcidoidea**

These small or very small parasitic forms have the wing venation very strongly reduced. Even the comparatively generalized *Spilochalcis* (fig. 13) in which Burks (1938) found enough venation to be able to determine the homologies, is much reduced, having lost two intercubitals and both recurrent nervures. Some very small forms have lost the venation entirely, and have fringed wings.

Superfamily **Proctotrupoidea**

The wings of this group of parasites have extremely reduced venation, with the hind wings lacking venation entirely. Figure 15 illustrates with wings of a rather large member of the group (*Pelecinus*) which has comparatively generalized wing venation. Extreme reduction is exhibited in most of the numerous small forms.

Superfamily **Cynipoidea**

Considerable reduction in venation is shown by even the generalized cynipoids, as shown in the wings of *Ibalia* (fig. 14). The venation is strongly crowded toward the front margin of the wing. Certain species of *Cynips* have venation differing in no great degree from that of *Ibalia*.

Division **Aculeata**

The more primitive superfamilies of the Aculeata are parasitic, like their terebrantian ancestors. It is easy to trace the steps leading from the parasitic mode of existence to the predaceous mode characteristic of most of the aculeates. The parasitic forms lay their eggs in or on the host, leaving the host to continue its existence as well as it can, and leaving the young parasite subject to the same environmental vicissitudes as the weakened host. The predaceous aculeata wasps differ only in that the host is paralyzed or killed, and placed in some protected situation in which it is kept safe as food for the developing

larva of the wasp. Some wasps only stow the prey into a natural crevice, or into the burrow of the prey; others construct a shelter for the larval food supply.

The transition between the zoophagous wasps and the pollen-eating bees is not difficult to explain from the purely nutritional standpoint. However, the transition from the point of view of behavior, and from the point of view of the origin of the pollen collecting structures, has not been satisfactorily explained. One difficulty is introduced by the fact that the two most primitive groups of bees—the colletids and prosopids—have different methods of collecting pollen, the colletids carrying it on brushes on the hind legs, the prosopids carrying it in the anterior portion of the digestive tract. I think it more likely that the last is the primitive method, with external brushes and corbiculae being added later, usually supplanting the digestive tract as a pollen-carrier. The evolutionary origin of the bees presents an interesting problem, one which might be solved by a comparative study of the structure and biology of certain southern hemisphere bees and wasps.

Some masarine wasps feed a mixture of pollen and nectar to their larvae, but the latter is apparently the most important constituent. Other masarine wasps feed their young with insects.

Superfamily **Bethyloidea**

Rhopalosoma (fig. 16) is taken as a generalized bethyloid wing, although the fusion of C, Sc, and R is a specialized feature. All the remaining bethyloid families (as do all the rest of the aculeate Hymenoptera) have C separate from Sc + R, leaving a costal cell. Except for this feature, the wing venation of ***Rhopalosoma*** is little reduced, only one of the intercubitals being lost, although the second recurrent nervure is represented only weakly. A jugal lobe is present in the hind wing.

Superfamily **Chrysidoidea**

Reduction in wing venation is far advanced in the chrysidids, involving the loss of the second recurrent nervure and all of the intercubitals. Rs^1 is extremely short, Rs + M almost or quite joining the prestigma. In the hind wings only faint indications of most of the longitudinal veins are present; a long, well-marked jugal lobe is present.

Superfamily **Scolioidea**

The front wing of *Scolia* (fig. 18) shows some specialization with respect to loss of an intercubital vein and a recurrent nervure, and the degeneration of the pterostigma, but other scolioids show no significant departure in venation from the basic clistogastran type. Sexual dimorphism in the hind wing of ***Myzine***, a tiphiid wasp, is shown in figures 19 and 20. In the Scolioidea, loss of wings has occurred in the females of several groups, and it may be that the primarily terrestrial hunting habits of the female of this superfamily has made possible the degeneration of the wings in these groups. It seems that where there is sexual dimorphism in wing venation, that the males have venation more like that of the primitive type, and that the females have the

venation more specialized or degenerate. In the scoliids there are closely spaced longitudinal ridges and grooves in the apical membrane of the wings; this apparently strengthens the wing membrane, and the venation is restricted much more to the basal region of the wings than in the tiphiids, which lack the apical striations.

Of the Mutillidae, the subfamily Brachycistinae is taken as the primitive type. These forms have a jugal lobe in the hind wing, and it is assumed that the absence of this lobe in the Mutillinae is secondary. The brachycistine venation (fig. 21) shows no significant departure from the generalized clistogastrous type, although the marginal cell is unusually abbreviated.

The slightly appendiculate marginal cell in *Brachycistis* brings up the question of the identity of the appendicular vein. The truncate and appendiculate type of marginal cell, which occurs quite frequently (*e.g.*, fig. 25), could be explained by assuming that R_1 has been pulled back away from its fusion with the costal margin of the wing, and that the appendiculation is the continuation of Rs beyond its juncture with R_1. However, in various groups Rs shows all degrees of curvature towards the costal margin, and in some becomes quite angulate. There seems to be no doubt that in these the entire lower and apical boundary of the marginal cell is made of Radial sector. I think the most likely explanation for the origin of the appendicular vein is that it is an accessory vein developing independently at the angulation of Rs.

An extra vein also occurs in the apical region of the front wing in *Dasymutilla* (fig. 22) and in some other mutillines. If it be assumed that the lower boundary of the third submarginal cell in this wing is formed by the strongly angulate Media, then the appendiculation of the submarginal cell is the continuation of Media. This interpretation, however, leaves the vein extending apically from the lower corner of the cell unidentified. It could possibly be a branch of M, but it would be surprising to see such a primitive feature occurring in an isolated fashion well up in the evolutionary scale in the Hymenoptera. Other species of *Dasymutilla* than the one figured show an additional extra vein extending apically from the middle of the third discoidal cell. This extra vein, and the one extending apically from the third submarginal cell should probably be considered to be accessory veins.

Superfamily **Formicoidea**

A very great amount of diversity exists in the venation of this group; only a relatively generalized type (*Eciton*, fig. 24) is illustrated here. In some primitive ants, the hind wing is lobed. *Eciton* shows reduction in the loss of an intercubital vein and of the second recurrent nervure.

Superfamily **Vespoidea**

Specializations exhibited in the wings of the family Vespidae, the only member of the superfamily, do not involve great changes in the venation. The front wings are generally folded lengthwise in repose (not so in *Euparagia*), and in some (Vespinae) the hind wing secondarily lacks a jugal lobe. One of the intercubitals (Rs) is sometimes absent.

Superfamily **Pompiloidea**

Pompilid venation (fig. 23) exhibits little departure from the hypothetical generalized clistogastran type. Rs is given off far basad of the pterostigma in the numerous genera studies.

Superfamily **Sphecoidea**

Wings of the sphecoid wasps often show no reduction in venation (fig. 26), but sometimes as many as two of the intercubitals are lost (Rs and 2 r-m, as in *Solenius*, fig. 25), and the second recurrent nervure may be lost. Hind wings of several genera secondarily lack jugal lobes.

Superfamily **Apoidea**

As in the sphecoids, reduction in venation is not the rule (fig. 27), and when it occurs, involves the loss of an intercubital (rarely two). In the subgenus *Diandrena* (of *Andrena*) it is obvious that is 2 r-m which has been lost; in *Prosopis*, the identity of the lost vein is not so obvious, but I think it also to be 2 r-m, and the same probably holds true for the other genera with two submarginal cells. Great reduction in venation is shown by *Trigona*, in which 2 r-m is faintly indicated, and 3 r-m is absent. All genera except *Bombus* and *Euglossa* have the hind wing lobed, with usually both the jugal and vannal lobes present. In forms like *Anthophora*, where the wings are strongly papillate apically, the venation is strongly shortened, being concentrated in the basal portion of the wing.

IV. SUMMARY

1. The plan of wing venation which would serve as an ancestral type for the suborder Clistogastra can be derived from the generalized hymenopterous wing proposed by Ross by only a slight reduction in the number of wing veins.

2. This ancestral pattern of wing venation persists little modified through the line of evolution leading to and including the advanced, powerfully flying bees and wasps.

3. The obvious changes in wing structure in the line of evolution leading to the bees and wasps involve the development of lobes on the trailing edge of the hind wing.

4. Extreme reduction of venation, resulting sometimes in complete loss of the venation, occurs characteristically in the small Terebrantia. Fairly strong reduction in venation is also characteristic of the larger parasitic Terebrantia; this specialization does not give them strong powers of flight as compared to the bees and wasps.

5. The ancestral hymenopteran presumably had phytophagous larvae. The insect had an ovipositor adapted for piercing plant tissues.

6. At least one parasitic group evolved from the phytophagous Hymenoptera, possibly by way of forms feeding on wood-boring insects. This parasitic group gave rise to the Terebrantia, which parasitize insects and spiders; one primarily gall-making group represents a secondary development of phytophagy.

7. From generalized Terebrantia, a group of predaceous Hymenoptera evolved by only a slight modification of the parasitic habit,

which involved immobilizing or killing the host and storing it in a protected place. This group gave rise to the wasps, bees, and ants.

8. The pollen- and nectar-feeding bees arose from the primarily predaceous wasps, but acceptable theories as to the transition between the two modes of life have not been worked out.

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A SELECTED BIBLIOGRAPHY OF THE INSECTS OF THE WORLD ASSOCIATED WITH SUGAR CANE, THEIR PREDATORS AND PARASITES, by J. S. WADE. 114 pages. International Society of Sugar Cane Technologists, Memoir No. 1, Honolulu, Hawaii. 1951.

This extensive list of references, in highly condensed but apparently quite usable form deals with an astonishing total of 1,277 species of insects chosen from a list of almost twice that number (2,366 species!) which are associated in one way or another with sugar cane.—M. T. J.

GAFANHOTOS EM SANTA CATARINA, by A. D. FERREIRA and FELIX SCHAEFER. 57 pages, 33 figures, 2 maps. Secretaria da Viação, Obras Públicas e Agricultura do Estado de Santa Catarina, Serviço de Defensa Sanitária Vegetal, Bulletin 4. Florianópolis, Brazil. 1951.

This work deals with the migratory locust, *Schistocerca cancellata* Serv. in the state of Santa Caterina and with the campaigns of 1946–47, 1947–48, 1948–49 against this insect. It is written in Portuguese.—M. T. J.

THE HOUSE FLY, ITS NATURAL HISTORY, MEDICAL IMPORTANCE, AND CONTROL, by LUTHER S. WEST. xvi+584 pages, 176 figs., frontispiece. Comstock Publishing Company, Ithaca, New York, 1951. Price, $7.50.

The house fly, one of our best-known, most widely distributed, and most dangerous insects, has been the subject of an immense amount of literature; yet there has been no comprehensive treatment in the English language since the appearance of the now classical works by Howard in 1911 and Hewitt in 1914. Entomologists and sanitarians will therefore welcome the publication of this well-prepared volume. The author, for more than twenty-five years, a student of the muscoid flies, has recently become more conspicuous in the field through his contribution to the *Manual of Tropical Medicine*, by Mackie, Hunter and Worth (1945). The present work has largely engaged his research efforts since the close of the war. The carefulness of the preparation and the thoroughness with which the literature has been searched are clearly evident in the finished product.

The book does not deal entirely with *Musca domestica*, though that species is the center of all discussion and the focus from which ramifications radiate. Some chapters, such as the one on myiasis, deal largely with flies of other families, though in this case special emphasis is given to the role of the housefly in this respect. It will probably come as a surprise to many who are not muscoid specialists to learn that the genus *Musca*, in the broader sense as accepted today comprises 204 named species, from one-fourth to one-third of which are valid. It probably will also be a surprise to many to learn that such large gaps still exist in our knowledge of some of the fundamental aspects of the biology of so familiar an insect. For example, the questions as to what constitutes the actual food of the larvae (*cf.* p. 183) and as to how the insect hibernates (*cf.* p. 118) not to mention the complex problems dealing with the development of resistance to insecticides, are still unsolved. On the other hand, the fact that a book of this magnitude, as strongly condensed and as selective in subject matter as it is, can be written about this insect, is a monument to mankind's research in the field.

The book can be used by the trained entomologist or sanitarian, even though he has no special knowledge of the muscoid flies. The basic fundamental information necessary for effective usage, including that leading to a morphological and taxonomic background, is well given. All important aspects of fly biology, as well as material covering the economic aspects of the fly, rearing and laboratory techniques, and emergency and planned control are included. It is noteworthy that a chapter can be devoted to the fly as a beneficial insect. The same insect that may cause high infant mortality and as high as fifty percent blindness (through fly born trachoma) in certain localities (*cf.* page vii), as well as widespread illness throughout much of the world, may under certain circumstances have beneficial larval habits or may serve as a useful laboratory animal.

Dr. West's book is well written, well illustrated, and presents a very good appearance in both the text and the illustrations. Typographical errors are few. Variation in nomenclature might cause some confusion; for example, the same fly, in different parts of the text, is called *Lucilia sericata* and *Phaenicia sericata*, and on page 189, the average reader will not realize that the discussion of *Philaematomyia insignis* and *Musca crassirostris* refers to the same insect. In most cases, such variation is obviously due, however, to the usage in the original sources from which the author obtained his material. The reference on page 144, to *Muscina stabulans* as "the true stable fly," is contrary to general usage. On page 145 the wing of *Fannia canicularis* is incorrect. Some errors have crept into Table 16 (pp. 320–325); on page 321, *Synthesiomyia nudiseta* is incorrectly referred to the Calliphoridae; on page 322 the geographical distribution of *Hypoderma bovis* is given as "Central and Southern Europe" (it is correctly given as "chiefly holarctic" in the preceding page); and on page 323 *Sarcophaga cooleyi*, a nearctic species, is given as "Warmer Palearctic." Finally, we might question the number of "Valid" *Musca* species, particularly in the New World. But all in all, we can commend our colleague for a fine and timely piece of work.—M. T. J.

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